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Tropical Medicine and Hygiene

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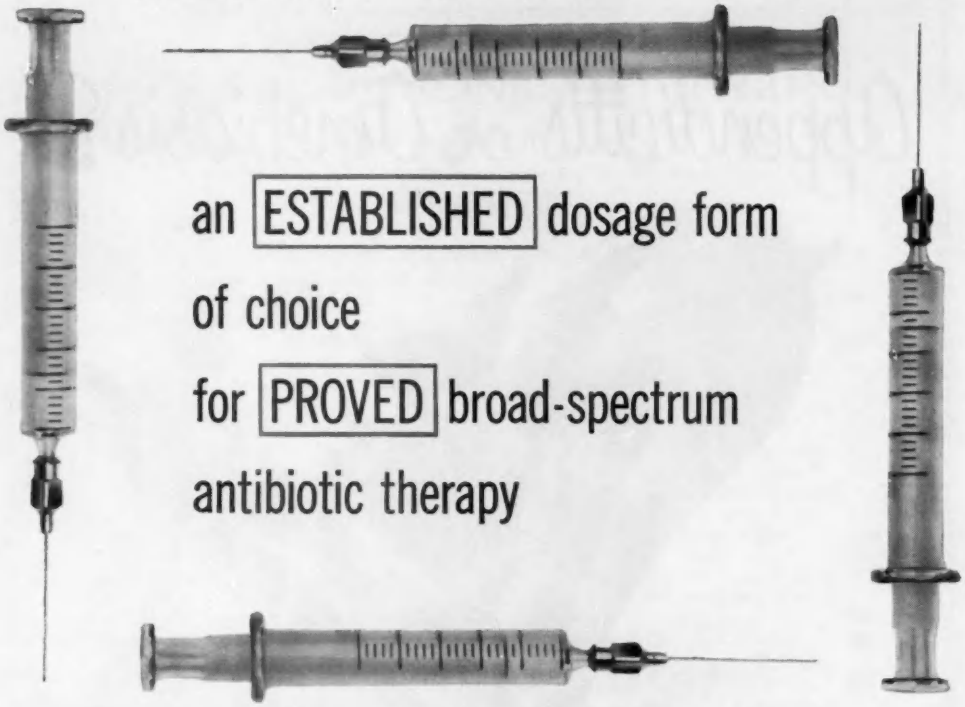

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1. Stempien, S. J., Marcus, D. M., and Kelso, R. W.: California Med., 74:36, Jan., 1951.
2. Sodeman, W. A., and Beaver, P. C.: Am. Jour. Med., 12:440, Apr., 1952.
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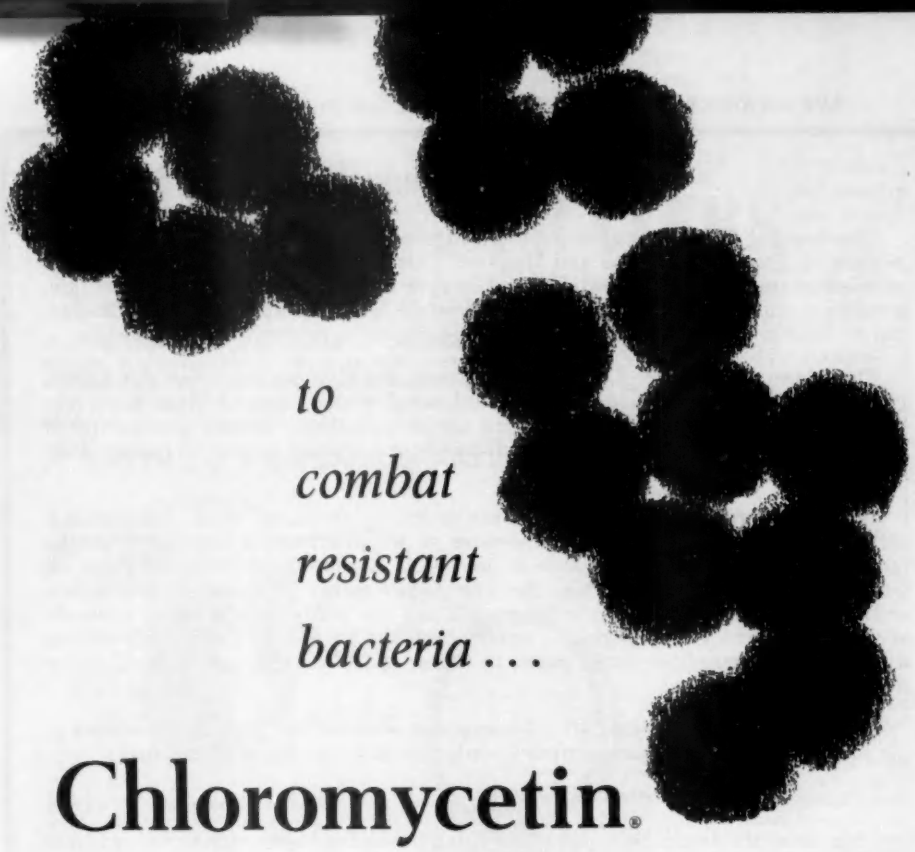

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*Pratt, R., & Dufrenoy, J.: Texas Rep. Biol. & Med. *12*:145, 1954.

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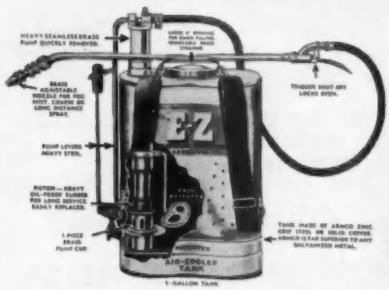

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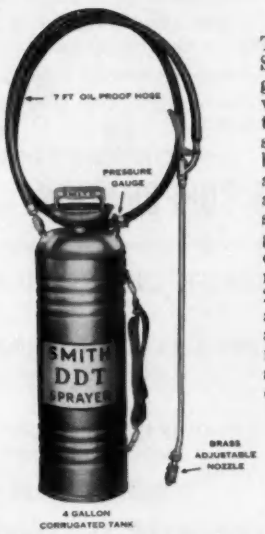

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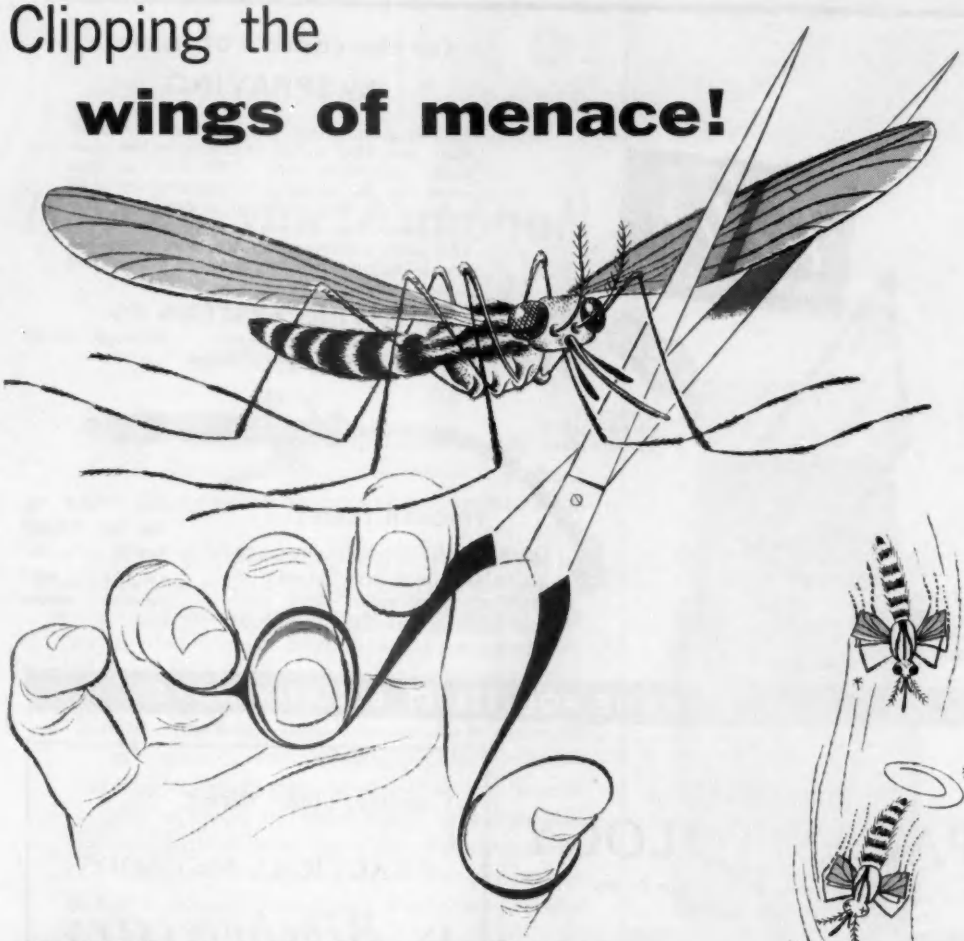

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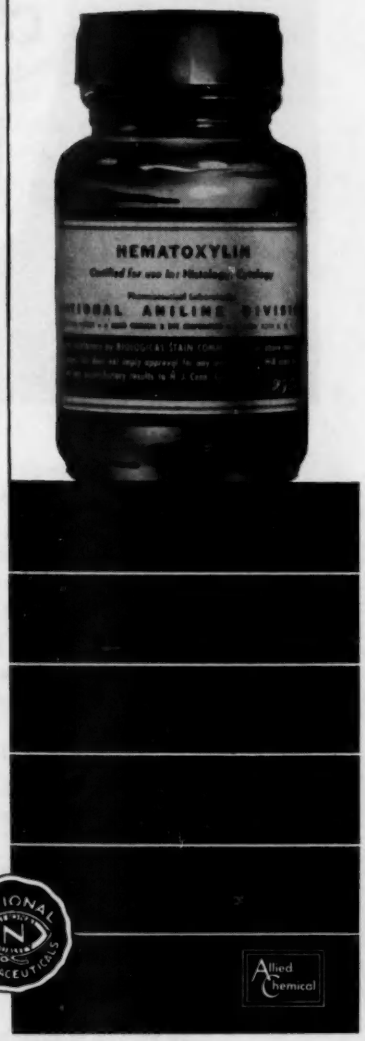

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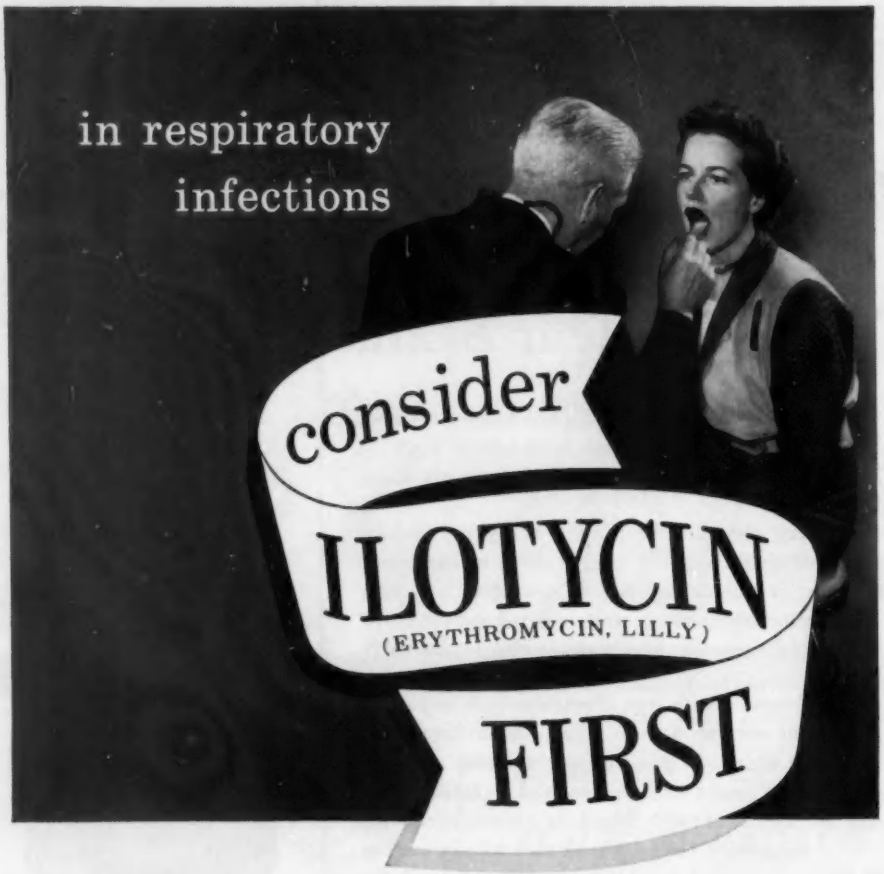

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Presidential Address

FREDERICK J. BRADY

It has been an honor to serve as President of the Society for the past year. During this year, I have been fortunate in having the able assistance of the officers, councillors and committee members, all of whom have made great contributions to the Society. Many of these officers have unstintingly devoted a great amount of their time to Society affairs.

Traditionally, tropical medicine has been at the forefront of medical programs. The first pathogenic agent described by science was a fungus, *Achorion schönleini*, which causes a disease that occurs today almost only in the tropics. Probably the first epidemiological study using modern inductive reasoning was Snow's study of cholera in London and its relation to the Broad Street Pump. Studies on host-parasite relationships, on transmission of disease by intermediate hosts, on the role of reservoir hosts were mostly in the realm of tropical medicine. The development of active immunizing agents was mostly in the field of tropical diseases. More recently, the finding of very effective synthetic compounds against the plasmodia, filarias and other parasites of man were real milestones in medical science.

However, these are accomplishments of the past. Today, evaluation of our programs is not based upon past accomplishments but upon how we meet today's needs. We do not justify our work with the knowledge that the Panama Canal could not have been built without control of yellow fever and malaria nor that the discovery of Bayer 205 as a therapeutic agent for trypanosomiasis was important in determining the fate of Germany's African colonies after World War I.

When control programs are designed to meet health needs, many factors must be considered such as mortality and morbidity from various diseases, popular acceptance of programs, and availability of effective measures. In research support more stress is given to those diseases of adjudged importance for which we do not have adequate preventive or control measures.

In light of these considerations let us examine the mortality rates for the United States. Heart disease and cancer account for two-thirds of all deaths. Of the communicable diseases, influenza and pneumonia are the sixth ranking cause of death and tuberculosis the tenth. The diseases generally regarded as tropical are negligible among the causes of death. In view of the lack of deaths from tropical disease in the United States, it is inevitable that this group of diseases should receive secondary attention. Furthermore, it is proper that health administrators place emphasis on the major killers.

However, there are many reasons why interest in exotic diseases should be sustained. These diseases affect our lives in an indirect way. Many groups recognize the present and potential future importance of tropical disease.

I would like to stress the increasing emphasis that our Government is giving to the betterment of health abroad as part of our foreign policy. Economic and social development of friendly nations is recognized as a prerequisite to the maintenance of world peace and security. Such development includes the control of debilitating diseases that waste human resources. It includes the development of personal friendships among kindred technicians who are able to work side by side without political differences. The Society can be proud that many of its members are actively engaged in these activities abroad under the aegis of our Foreign Operations Administration, the World Health Organization, and the Pan American Sanitary Bureau.

In referring to the value of health programs in our foreign policy, Assistant Secretary of State David Key stated:

> "The psychological or spiritual contributions of WHO to world peace are less tangible than the economic contributions and so far have received far less attention. I am convinced, however, that in the long run they are more fundamental, for they deal with men's minds. WHO, like other specialized agencies, is promoting democratic and not totalitarian ways of life. It is promoting self-respect, self-reliance, and freedom for the individual."

Secondly, the United States has in recent years become increasingly dependent upon the tropics for supplies of materials that have become essential to our welfare. Health development helps insuring the free world's supply of these essential products. The Editor of Tropical Medicine News in the issue of April 1951, pointed out the almost complete dependence of the U. S. on nations abroad to supply us with tin, chrome, rubber, and manganese.

Thirdly, with the continued expansion of foreign trade and foreign investments, our stake in health abroad is assuming greater financial importance. Both trade and investment have health implications. It is noteworthy that U. S. investments abroad, direct and indirect, private and governmental, are now about $40 billion. These investments have expanded at a rapid pace. In the three year period of 1950–1952, direct private investment in Africa and Asia increased by more than 65%. This rapid increase will continue and some economists predict severalfold increases in a few years.

A recent illustration shows the effect that health matters may have on world trade. Recently there was an outbreak of yellow fever in Trinidad, which by October totalled 12 confirmed cases and three deaths. Even though we ignore the humanitarian aspects of this outbreak, the economic impact was startling. Trinidad in a sense was quarantined by much of the world with extreme dislocation of trade and travel. The island was avoided by air and surface craft; at least one country barred direct travel. Someone estimated that the cost to Trinidad might amount to $23,000,000—a staggering figure indeed for a small country to pay for less than a score of cases of this disease.

Fourthly, as has often been pointed out in the past, faster travel has decreased the value of traditional quarantine measures by putting remote areas of the world within two-days flight range of our frontiers. Continued quarantine vigi-

lance is a palliative remedy and not a cure. There is a growing realization that several pestilential diseases can and should be eradicated from the areas of the world where they continually threaten international travel.

It is heartening that within the month representatives of 23 nations voted without dissent to authorize the expenditure of $144,000 for control of smallpox in the Americas. These same nations pledged $100,000 to begin a systematic campaign directed towards malaria control and agreed to ask their governments to increase the budget of the Pan American Sanitary Bureau by a similar amount in future years for this activity.

Fifthly, our countrymen are traveling abroad more than ever before and thus risk more exposure to tropical diseases. In 1952, almost 800,000 civilians traveled abroad not including travel to Canada and Mexico. This year the number of travelers may exceed the million mark. Not only is travel to be considered but large numbers of Americans are stationed abroad for variable intervals either in military or civilian capacities. Some economists have estimated that as many as one of every four college graduates 25 years from now will find his employment abroad in Africa, Asia, or South America.

Lastly, I would remind you of the necessity that the United States maintain a corps of personnel well trained in tropical medicine in case of future emergencies. You all recall the lecture of General Phillips at last year's Academy symposium wherein tactical military difficulties due to tropical diseases was discussed. Malaria early in World War II proved to be of major military importance in such places as New Guinea where up to 90 per cent of companies at the front were rendered unfit as fighting men. Schistosomiasis and filariasis became of major military importance even though they had been adjudged to be of negligible military significance only a year earlier. Even kala-azar was contracted by substantial numbers of troops but in many cases was not diagnosed until one or two years had elapsed.

These are some of the reasons for our support of tropical medicine. They are all interests not immediately apparent but nonetheless are of great importance to our welfare.

It is an honor indeed for me to have served as your President. It is with complete confidence in our future that I turn over the leadership of the Society to the new President, Dr. Taliaferro.

RECENT ADVANCES IN OUR KNOWLEDGE OF DENGUE AND SANDFLY FEVER[1, 2]

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In the first years of the 20th century Charles Franklin Craig together with Ashburn performed the first fundamental experimental studies on dengue fever in the Philippines and established the viral etiology of this disease (Ashburn and Craig, 1907). I am grateful for the privilege of summarizing recent advances in our knowledge of dengue and of another arthropod-borne virus disease, i.e., sandfly (pappataci, phlebotomus) fever, in a lecture designed to honor Col. Craig's name. Dengue fever and sandfly fever do not have very much in common. They are endemic in different parts of the world, they are transmitted by different vectors and are caused by totally different and unrelated viruses. The only reason they are being discussed together is that during World War II it became my lot to study both of these diseases and their etiologic agents, and ever since 1943 one or another or both of the entities have engaged at least a portion of my interest and effort. Together with an ever-changing parade of able and industrious young co-workers, it has been possible year by year to learn a little bit more about these interesting infectious diseases and the agents which cause them.

DENGUE

Prior to 1944, experimental work on dengue was carried out sporadically because it could be done only in human volunteers and any virus that was used disappeared when a particular investigation was finished. To begin our own systematic studies it was necessary first of all to obtain blood from patients who, on clinical and epidemiologic grounds, were assumed to be suffering from dengue, then to transmit the infection in series to human volunteers by inoculation as well as by *Aedes aegypti*, to prove that it was caused by a filterable virus different from other known viruses—and the agent so recovered and characterized and preserved in the frozen or lyophilized state was called dengue virus. Agents with similar properties were recovered from epidemics in Hawaii, New Guinea, India and Japan (Sabin, 1952).

Many interesting new facts, summarized elsewhere (Sabin, 1948, 1950, 1952, 1952a), emerged from the studies between 1944 and 1952. The following synthe-

[1] The Nineteenth Annual Charles Franklin Craig Lecture, delivered before the American Society of Tropical Medicine and Hygiene, November 5, 1954.

[2] The studies summarized in this lecture were sponsored by the Commission on Virus and Rickettsial Diseases, Armed Forces Epidemiological Board, and supported partly by the office of The Surgeon General, Department of the Army and partly by funds provided under Contract AF 18(600)548 with the USAF School of Aviation Medicine, Randolph Field, Texas.

sis of our knowledge at that time may be helpful in understanding the need for the subsequent studies to be summarized in this lecture. Dengue is caused by at least two immunologically distinct but antigenically related viruses. Although the ten currently available strains derived from Hawaii, New Guinea, India and Japan all belong to the two types, future field studies must take into account the possibility that additional immunologically distinct types of dengue virus may occur in nature. Homotypic immunity persists for years under conditions which preclude reinfection. The clinical manifestations resulting from reinfection with a heterotypic dengue virus may be absent, negligible, or unrecognizable as dengue fever depending on the interval between the first and second infections. Mosquitoes feeding on individuals with these negligible or clinically modified, mild, rash-free infections can give rise to clinically characteristic and severe dengue fever when they bite human beings without previous exposure to any dengue virus.

Naturally occurring human dengue virus multiplies very poorly, and only rarely with associated clinical manifestations, in the nervous system of adult mice, but more extensively and with associated paralytic manifestations in the nervous system of newborn mice. Continued passage in mice (newborn or adult) results in the emergence of mutants with ever-increasing capacity for multiplication in the nervous system of newborn and adult mice, and with different pathogenic properties in human beings and monkeys. The high-titered Type 1 and Type 2 dengue viruses obtained from the brains of suckling mice not only greatly facilitated the performance of neutralization tests in adult mice but also permitted the preparation of potent complement-fixing (C-F) antigens. Studies on human and monkey sera then revealed that the neutralizing antibody response is generally much more type-specific than the complement-fixing antibody. Thus, sera which contained only homotypic neutralizing antibody usually contained heterotypic complement-fixing antibody as well. Studies on antigenic relationships with the aid of concentrated C-F antigens furthermore revealed that the dengue viruses had some very interesting and unexpected relatives. Thus it was found that the viruses of dengue, yellow fever, West Nile fever and Japanese B encephalitis were antigenically related. This relationship is of such an order that infection with the dengue viruses only rarely results in the development of small amounts of neutralizing antibody for the West Nile and Japanese B viruses, but the fact that this can occur must be kept in mind in any evaluation of serologic survey data as an index to the occurrence of members of this group in certain parts of the world. This antigenic relationship is also not associated with any significant active cross immunity. Thus immunization of human volunteers with the 17-D strain of yellow fever virus produced no resistance to even small amounts of dengue virus.

Virus derived from the first 6 passages of the Type 1 dengue virus (Hawaiian strain) in mice produced varying clinical manifestations in human volunteers, but beginning with the seventh mouse passage a mutant emerged which had lost its capacity to produce the febrile systemic illness but retained its capacity to produce a rash and solid immunity to the unmodified virus (Sabin and Schles-

inger, 1945). Further tests on human volunteers with 15th and 19th mouse passage virus established its innocuousness as well as the fact that a single mouse-infective dose was sufficient to produce immunity in human beings (Sabin, 1952). Since infection with unmodified human virus produced only homotypic long-lasting immunity, it was clear that immunization with one type of the mouse-adapted virus would not confer long-lasting protection against dengue fever caused by another type. However, it was not until 1951 that strains of the Type 2 dengue virus were thoroughly adapted to mice (Meiklejohn, England and Lennette, 1952; Schlesinger and Frankel, 1952) but their behavior in human beings was still unknown.

Intracerebral injection of human dengue virus and of early passage levels of mouse-adapted virus in rhesus or cynomolgus monkeys produces an inapparent infection with focal infiltrative lesions in the brain stem and spinal cord. However, after a large number of intracerebral passages in mice the intracerebral injection in monkeys of the resulting larger concentrations of virus occasionally resulted in a paralytic disease which clinically and histologically was not readily distinguishable from that of poliomyelitis. The available data did not permit a decision as to whether this paralytogenic activity represented a qualitative change due to the appearance of a new mutant or was merely the result of the inoculation of very much larger amounts of virus. The answer to this question would naturally have a determining influence on the use of such virus preparations for active immunization of human beings.

In 1952, Dr. Benjamin H. Sweet and I undertook a new series of studies dealing with the prevention and diagnosis of dengue. The important new findings will be summarized here and the detailed results will be published elsewhere. Intracerebral titration of the 118th mouse passage Hawaiian, Type 1 dengue virus revealed that more monkeys developed paralysis after inoculation of smaller doses than of larger doses; thus, 5 of 15 rhesus monkeys inoculated with 400 to 40,000 mouse LD_{50} developed paralysis, while only 1 of 15 became paralyzed after inoculation of 4,000,000 to 40,000,000 mouse LD_{50}. Since a similar test with the 15th mouse passage of the same strain of virus produced no paralysis in any of 30 monkeys inoculated with amounts varying from 1 to 100,000 mouse LD_{50}, it was concluded that a new mutant was responsible for the paralytogenic activity of the later mouse passages. The 101st mouse passage of this strain, which had been submitted to 91 serial passages in chick embryos by Dr. R. Walter Schlesinger (1950), was also titrated in monkeys, and the results showed that the new mutant was not eliminated during the course of continued propagation in chick embryos. Similar tests in 37 monkeys inoculated with 8 to 80,000,000 LD_{50} of the 18th mouse passage of the New Guinea "C" strain of Type 2 dengue virus revealed no paralytogenic activity. The same preparation of 18th mouse passage Type 2 virus was tested in 4 human beings who received a subcutaneous injection of 0.3 ml. of a 1 per cent centrifuged extract of mouse brain ($10^{6.4}$ mouse LD_{50}). Aside from a rash, there were no other clinical manifestations of infection; all 4 developed homotypic neutralizing antibodies and 3 also developed heterotypic neutralizing antibody in lower titer.

Although the 17-D strain of yellow fever virus, which has been proved to be harmless in millions of human beings, produces encephalomyelitis in a certain proportion of intracerebrally inoculated monkeys, it appeared more desirable to use only those mouse passage levels of dengue virus which are devoid of the paralytogenic mutant as fixed seed lots for the preparation of vaccine for human use. It became necessary to determine, therefore, whether or not a certain number of additional passages in mice could be made from these fixed seed lots without encountering the paralytogenic mutant. After 5 additional passages of the Type 1 virus and 8 additional passages of the Type 2 virus, tests on large numbers of monkeys revealed no evidence of paralytogenic activity. Accordingly, there would appear to be enough "genetic stability" in these early passage levels of both types of the mouse-adapted dengue virus to permit their use for vaccine production, although each lot will have to be checked by the intracerebral injection of at least 10 monkeys with different amounts of virus.

Studies were also carried out to determine whether these early passage levels of high-titered, mouse-adapted virus could be propagated in chick embryos or monkey kidney tissue cultures. The Type 1, Hawaiian strain (mouse passage 18) could be passed in series in 5-day old chick embryos, but after 5 passages the level of viral multiplication was still minimal and only about one millionth of that achieved in the brains of suckling mice. The Type 2, New Guinea "C" strain (mouse passage 21) despite its high level of multiplication in the brains of suckling mice (titers of 10^{-7} to 10^{-8} per 0.03 ml.) completely failed to multiply at a detectable level in the chick embryos. Both types of dengue virus multiplied in tissue cultures of monkey kidney without producing a cytopathogenic effect. In the third tissue culture passage the Type 2 virus yielded fluids with $10^{6.2}$ mouse LD_{50} per ml. which, however, is only $\frac{1}{50}$ the amount of virus found per ml. of 10 per cent extract of suckling mouse brains. The Type 1 virus, on the other hand, multiplied at a much lower level—$10^{3.3}$ to $10^{3.8}$ mouse LD_{50} per ml. of culture fluid which is only about $\frac{1}{1000}$ of that found per ml. of 10 per cent extract of suckling mouse brain. It is possible that further serial passages in monkey kidney tissue cultures may increase the level of viral multiplication and thus provide a satisfactory alternative source of virus for vaccine production.[3]

Next I should like to summarize some new data on the use of serologic tests for diagnosis and epidemiologic survey. Utilizing the techniques which we had developed for the recovery of hemagglutinins from many of the arthropod-borne viruses (Sabin and Buescher, 1950; Sabin, 1951; Chanock and Sabin, 1953 and 1954), it proved possible to extract hemagglutinins for chick erythrocytes from the brains of suckling mice infected with the dengue viruses. The two types of dengue hemagglutinin possess distinctive pH and temperature requirements for optimum reaction with chick erythrocytes and can be identified by their reaction with type-specific sera. The technique for their preparation, their physico-

[3] Since delivery of this lecture, Dr. Benjamin H. Sweet and I have succeeded in Selecting variants which multiply at higher levels in monkey kidney tissue cultures but still without cytopathogenic effect. The Type 1 dengue virus now yields 10^{6} to $10^{6.5}$ mouse LD_{50} per ml. and the Type 2 virus $10^{6.5}$ to $10^{7.4}$ mouse LD_{50} per ml. of culture fluid.

chemical properties and reaction with antibody resulting in inhibition of hemagglutination were recently described in detail elsewhere (Sweet and Sabin, 1954). Dengue antisera which are strictly homotypic by the neutralization test react to varying titers with heterotypic dengue hemagglutinin. By means of the hemagglutination-inhibition test the dengue viruses were found to be antigenically related not only to the viruses of yellow fever, West Nile fever and Japanese B encephalitis but also to the virus of St. Louis encephalitis; no relationship was found with the viruses of western equine encephalitis, sandfly fever and Rift Valley fever.

To help determine which of the available laboratory tests would be the simplest and at the same time most useful for the practical specific diagnosis of dengue, as well as to provide a pattern of the antibody response which would aid in the interpretation of data that may be obtained in the field, 4 patients were experimentally infected with Type 1 or Type 2 human dengue virus and serial specimens of their serum were tested for homotypic and heterotypic dengue antibodies as well as for the related West Nile fever and Japanese B encephalitis antibodies (Sweet and Sabin, 1954).

These tests showed that: 1) the homotypic neutralizing and hemagglutination-inhibiting antibodies appeared before the complement fixing antibodies; 2) by means of the simple *in vitro* hemagglutination-inhibition test it was possible in each case to make a diagnosis 7 days after onset of fever, i.e., within 24 to 48 hours after the temperature had returned to normal; 3) it was necessary to use both Type 1 and Type 2 dengue antigens for diagnostic purposes since the heterotypic response may be delayed or of a lower order: 4) the greatest crossing occurred in the hemagglutination-inhibition tests with the West Nile fever antigen; in all patients there was at least one period sometime between the 2nd and 16th weeks after onset of fever when the hemagglutination-inhibition test would either have made it impossible to distinguish between infection with West Nile and dengue fever viruses, or an erroneous impression might have been gained as to the etiology of the infection because the West Nile titers were higher than the homotypic dengue titers; 5) the Japanese B encephalitis hemagglutination antigen also yielded high titers of antibody in these patients, in some instances at certain times after onset of infection almost as high as those of the homotypic dengue antibody but never as high as the titers reached with the West Nile antigen; 6) while some crossing was also observed in the complement-fixation test it was at no time of a degree which would have made it difficult to determine which virus was responsible for the infection.

On the basis of these results it is evident that in parts of the world where West Nile fever or Japanese B encephalitis may be encountered at the same time as dengue, it would be necessary to use both the complement-fixation and the hemagglutination-inhibition tests for the primary identification of the infecting virus in a given outbreak. As regards the confirmation of the diagnosis in the individual patient, hemagglutination-inhibition is the test of choice because this antibody appears much earlier than the complement fixing antibody and the diagnosis can be confirmed within a day or two after defervescence, i.e., before the patient has to be discharged from the hospital.

The usefulness of the dengue hemagglutination-inhibition test for epidemiologic survey was demonstrated in tests on sera from residents of Malaya and Borneo made available to us by Doctors Smadel and Pond of the Army Medical School. Ninety-five per cent of 20 Malayan sera and 90 per cent of 10 North Borneo sera contained antibodies for the Type 1 virus, for the most part in such high titers as to suggest recent infection. Since none had antibody for the Type 2 virus without invariably having it in a higher titer for the Type 1 virus, and because the incidence of positive tests for Type 2 antibody was only 75 per cent in the Malayan group and 70 per cent in the North Borneo group, it is possible that only Type 1 dengue virus was responsible for infection of these people.

The simple and rapid *in vitro* hemagglutination-inhibition test for dengue antibody also provides a useful tool for studies on vectors of these viruses and was so employed in a cooperative study with Doctors Rosen and Rozeboom (1954) to prove that *Aedes polynesiensis* can transmit human dengue virus from monkey to monkey.

The tools are now available for many interesting field studies designed to determine the areas of the world in which these viruses are endemic, to elucidate the question of a possible "jungle reservoir", and to establish whether or not more than 2 types of dengue virus are responsible for naturally occurring infections. Tests now in progress should indicate whether or not both types of dengue virus can be injected simultaneously in human beings without interfering with the development of adequate immunity to both.

SANDFLY (PAPPATACI, PHLEBOTOMUS) FEVER

Sandfly fever is endemic in those parts of Europe, Africa and Asia which lie between 20 and 45 degrees north latitude and harbor the vector *Phlebotomus papatasi*. It is usually not recognized as a distinct entity in the native populations because it is only one of a large variety of infectious agents which cause nondescript, mild febrile illnesses during infancy and childhood. It has long been recognized, however, as a disturbing epidemic disease among military personnel and other immigrants who come to endemic areas from regions that are free of the infection. The great effectiveness of DDT against *Phlebotomus papatasi* has provided an important tool in checking the transmission of the disease. While the laboratory investigations during World War II provided important information on the basic properties of the viruses encountered in the Middle East and Italy, no susceptible laboratory animal or method of propagating the virus outside the human body was discovered (Sabin, Philip and Paul, 1944; Sabin, 1951a and 1952b). However, the viruses, in the form of human serum, were successfully preserved in the frozen and lyophilized states for many years, and in 1952 a renewed attempt was made, in association with Dr. Benjamin H. Sweet, to adapt sandfly fever virus to mice. The successful results, first presented last year at the 5th International Congress of Tropical Medicine and Malaria in Istanbul, can be summarized as follows.

The Sicilian strain of virus, after 9 years of storage in the frozen state in the form of human serum, was successfully adapted to the brains of newborn mice. Three blind passages at 7-day intervals yielded a virus which produced en-

cephalitis in 2 to 4-day old mice after an incubation period of 11 to 12 days. At this stage the virus produced lesions predominantly in the hypothalamus and midbrain, but none in the muscles, fat, pancreas or other viscera. On continued intracerebral passage in newborn mice the incubation period gradually shortened, the level of multiplication gradually rose, and after the 10th passage the virus began to kill adult as well as newborn mice. By the 25th passage in the brains of newborn mice the virus had achieved a titer of 10^{-7} (per 0.03 ml.) in approximately 4-week old mice.

The identity of this highly potent mouse-adapted virus with the etiologic agent of Sicilian sandfly fever was proved by the fact that patients who had no neutralizing antibody for this virus during the acute phase of their illness, resulting from inoculation with sera brought back from Sicily in 1943, developed such antibody during convalescence. Neutralizing antibodies for this virus were also found in other patients, 4 to 5 months and even 9 years after a single experimental infection with human sandfly fever virus. Patients inoculated with dengue virus developed no antibody for the mouse-adapted sandfly fever virus. The sandfly virus was found to be different from the dengue, West Nile, Japanese B, St. Louis and yellow fever viruses in that it regularly killed adult "PRI" mice, which are genetically resistant to these other viruses (Sabin, 1952c).

Intracerebral injection of the highly pathogenic, mouse-adapted sandfly fever virus produced neither clinical signs of disease nor lesions in 10 rhesus monkeys, young rabbits, guinea pigs and hamsters. Inapparent infection may have occurred, however, because they developed antibody. Three patients were inoculated with the 10th mouse passage virus and three with the 20th mouse passage. None became ill, but neutralizing antibodies were found in their blood 3 weeks after inoculation. Six weeks after inoculation of the mouse-adapted virus, the six patients were tested for active immunity by an intravenous injection of unmodified human sandfly fever virus. All six were found to be immune, while two other patients who served as controls, developed typical sandfly fever and neutralizing antibodies for the mouse-adapted virus.

The mouse-adapted Sicilian virus yielded a C-F antigen of high potency which reacted specifically and in high titer with antiserum prepared in mice, but negative results were obtained with the convalescent sera of the experimentally infected patients. It is of special interest, therefore, that viruses recovered in suckling mice from the blood of 2 febrile patients in Egypt were identified by Doctors Richard Taylor and Jordi Casals as being immunologically identical with our Sicilian strain of virus (personal communication). Dr. Taylor, furthermore, informed me that the patients from whom these viruses were recovered developed readily demonstrable C-F antibodies not only for their own viruses but also for our Sicilian strain.

In an attempt to find still another diagnostic method for this viral infection we searched for a hemagglutinin, but the methods which were successful with the dengue and other mosquito-transmitted viruses yielded only small and insignificant amounts from the brains of suckling mice infected with the Sicilian virus. Systematic studies revealed that an inhibitor which could be extracted with

alcohol but not with acetone or ether was the interfering agent. Furthermore, unlike the hemagglutinins associated with the dengue, West Nile, Japanese B, and St. Louis viruses which do not react and are most unstable at 37°C., the Sicilian virus hemagglutinin reacted with chick erythrocytes optimally at 37°C. between pH 5.5 and 6.5, although it could also be demonstrated in lower titers at room temperature and 4°C., over a wide range of pH from 5.5 to 8.0. The other viral hemagglutinins just mentioned were unaffected by merthiolate (1:10,000) while the Sicilian virus hemagglutinin was completely inactivated. While acetone precipitation of serum yields fully active antibody fractions devoid of nonspecific inhibitors for the other viral hemagglutinins just mentioned, this technique failed in the case of the Sicilian virus hemagglutinin. The additional extraction with alcohol was effective with some sera but not with others, while the technique of Seitz filtration of the serum, proposed by Casals and Brown (1954) for other viral hemagglutinins, proved satisfactory. Antiserum prepared in mice yielded, after Seitz filtration, a hemagglutination-inhibition titer of 1:160 against 4 units of Sicilian virus hemagglutinin, while antisera against the Naples strain of sandfly fever virus, the dengue, West Nile, Japanese B, St. Louis, western and eastern equine, Russian spring-summer encephalitis and Rift Valley fever viruses completely failed to react. However, Seitz-filtered, human Sicilian virus convalescent sera yielded only very low titers or negative reactions. Further work, however, needs to be done along these lines since Dr. Sweet found that Seitz filtration markedly reduced the titer of hemagglutination-inhibition antibodies in dengue antisera.

The high-titered, mouse-adapted Sicilian virus could not be propagated in chick embryos and could not be carried beyond the first passage in monkey kidney tissue cultures.

The Naples strain of virus was recovered in 1944 from the blood of an American soldier with a clinically diagnosed case of sandfly fever in Naples by passage in human volunteers in the U.S.A. Although it possessed the properties of sandfly fever virus, the Naples strain proved to be immunologically distinct from the Sicilian and Middle East strains, and it could not be identified by the crucial test of transmission by *Phlebotomus papatasi* (Sabin, 1951a). In 1953, after 8 years of storage in the form of lyophilized human serum, the Naples virus was successfully passed in 2 patients, and the serum from one of these patients was used for the successful isolation of a mouse-pathogenic variant. A single, blind intracerebral passage in newborn mice yielded an agent which produced encephalitis after an incubation period of 14 days. After 5 consecutive intracerebral passages in 2 to 4-day old mice, the incubation period was reduced to 8 days, and although the intracerebral titer in these mice was $10^{-5.8}$ per 0.01 ml. it produced no signs in 4-week old mice. It was not until 35 consecutive intracerebral passages had been made in newborn mice that the virus also became regularly pathogenic for weaned mice. In its 55th passage the virus has a similar titer in newborn and weaned mice—still only about $10^{-5.9}$ although the incubation period is almost twice as long in the older mice. The Naples virus is similar to the Sicilian strain in that it regularly kills adult "PRI" mice which are genetically

resistant to the dengue viruses and their antigenic relatives (Sabin, 1952c). The mouse-adapted virus was identified with that in the human serum by appropriate neutralization tests. The complete lack of immunologic relationship between the Naples and Sicilian strains of virus, which was first found in human cross-immunity tests, was also found in neutralization and complement fixation tests with both human convalescent and mouse hyperimmune sera.

The Naples virus produces clinical manifestations in suckling and older mice after intracerebral and intranasal inoculation but not after subcutaneous or intraperitoneal injection. Neither lesions or clinical signs were observed in intracerebrally inoculated young rabbits, guinea pigs or hamsters. While the Sicilian strain produced neither lesions nor signs in intracerebrally inoculated rhesus monkeys, the Naples strain produced fever and infiltrative lesions of varying extent in all 10 monkeys which received either 10^6 or 10^4 mouse LD_{50}. No multiplication was demonstrable in monkey kidney tissue cultures. The mouse-adapted Naples virus still remains to be tested in human beings.[4] While a C-F antigen derived from this virus yields high titers with homologous mouse hyperimmune serum, no titers in excess of 1:2 have as yet been obtained with human convalescent sera.

The new data obtained on dengue and sandfly fever not only give us the tools for obtaining an even greater understanding of these two arthropod-borne virus diseases, but also the means whereby it may be possible to immunize against them when the need for such protection should arise.

SUMMARY

The chief advances in our knowledge of dengue during the past 2 years are in the demonstration that: 1) paralytogenic activity in intracerebrally inoculated monkeys of viruses submitted to prolonged passages in mice is due to the appearance of a new mutant and not to increased concentration of virus; 2) the Type 2, New Guinea "C" strain, after 18 passages in mice, has lost its virulence for man but not its immunogenic capacity; 3) early passage levels of both immunologic types of mouse-adapted virus possess sufficient "genetic stability" as regards propagation without appearance of the paralytogenic mutant, that fixed seed lots can be used for the preparation of a bivalent vaccine for human use; 4) specific hemagglutinins are associated with the 2 types of dengue virus and can be the basis of a simple *in vitro* serologic test for rapid diagnosis and epidemiologic survey.

The chief advances in our knowledge of sandfly fever during the past 2 years stem from the demonstration that mouse pathogenic mutants can be developed by propagation in newborn mice. The immunologically distinct Sicilian and Naples strains were both adapted to mice to a point where they are equally virulent for newborn and adult mice by the intracerebral route. Tests performed with

[4] Since delivery of this lecture, the 30th mouse passage of the Naples virus was injected intracutaneously in 4 human beings in a dose of $10^{5.9}$ mouse LD_{50}. No fever or other signs of infection appeared but immunity to subsequent inoculation of the unmodified human virus was demonstrated.

the mouse-adapted Sicilian strain in human beings revealed that it lost its virulence but not its immunogenic capacity. These mouse-adapted strains provide new tools for diagnosis, epidemiologic survey and prevention of sandfly fever.

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REVIEW OF INVESTIGATIONS IN MALARIA CHEMOTHERAPY (U. S. A.) 1946 TO 1954[1]

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The history of research in malaria chemotherapy in the period 1946 to 1954 was centered largely about investigations stimulated by the Research Grants program of the Public Health Service and later carried to the point of field evaluation under guidance of the National Research Council with active financial support and participation of the Armed Services. There were independent excursions in malaria chemotherapy by various pharmaceutical establishments and by university workers, but with the possible exception of the work associated with the discovery of pyrimethamine, none of these efforts was of great weight nor did they produce results which now command attention either at the practical or theoretical level.

The major research efforts in malaria chemotherapy of the post World War II era can be divided into two main periods. The first, 1946 to 1950, involved clarification, consolidation, and extension of leads developed during the program of malaria research directed by the Office of Scientific Research and Development (OSRD) during World War II. The second, 1951 to 1954, involved critical laboratory and field evaluations of the most promising curative agents developed during the period 1946 to 1950, and the laboratory evaluation of the potentialities of an entirely new type of antimalarial drug, pyrimethamine.

Investigations carried out during the early years of the OSRD malaria program had demonstrated the effectiveness of quinacrine as a suppressive and therapeutic agent and in particular had delineated the most effective ways of using the drug. During the intensive investigations which followed this development, several groups of compounds were uncovered which in stylized tests against avian malaria and preliminary trials against the human malarias seemed to possess suppressive and therapeutic qualities superior to those of quinacrine or quinine. These compounds included the 4-aminoquinolines, the most promising of which were chloroquine, amodiaquin, and oxychloroquine, the 4-quinolinemethanols, and a small group of naphthoquinones. To this assemblage were added the biguanides, and particularly chlorguanide, which had been revealed by the British malaria studies and made available to the OSRD investigators through the scientific liaison between Great Britain and the U. S. A. Although the effectiveness of all these compounds had been demonstrated in a general way at the termination of the OSRD program, none except chloroquine, a 4-aminoquinoline, had been evaluated to the extent where recommendations for general use could be considered.

[1] This paper was prepared at the request of the Division of Medical Sciences, National Research Council, and approved by its Panel on Malaria.

It had also been recognized, during the latter years of the World War II studies, that certain prewar investigations by British investigators had demonstrated that the old German drug pamaquine possessed a property not common to other antimalarial drugs, namely, an ability to cure naturally acquired *vivax* malaria. This recognition led to confirmatory studies and subsequently to a large program involving synthesis, pharmacological investigation, and clinical evaluation of the 8-aminoquinolines, a program which dominated the last year of the OSRD malaria activities. At the end of the War this effort had led to development of at least one compound, pentaquine, believed to be superior to pamaquine.

With the termination of hostilities, many of the investigators in the OSRD program renewed their interests in prewar scientific activities. Others maintained their interest in the problems of malaria chemotherapy, recognizing (a) that further work was needed in order to consolidate the positions of the most promising suppressive and therapeutic agents and (b) that further work was needed to develop a safe and generally useful drug which would effect radical cure of *vivax* malaria. These latter individuals felt that investigations could be pursued most effectively if use were made of the pattern of cooperative effort which had been established during the OSRD program.

The need, desirability, and opportunity for such an effort were brought to the attention of the Public Health Service which had just begun to organize its extramural research grants program. Approving the principles of the effort, the Public Health Service organized the Malaria Study Section. The initial membership[2] of this Study Section, largely individuals who had coordinated the activities of the OSRD malaria program, was in keeping with the objective of rounding out the chemotherapeutic studies of World War II. The Study Section implemented this objective by recommending grants-in-aid to chemists, pharmacologists, parasitologists, and clinical investigators who wished to participate in the extended program. The principal investigators, institutions represented, and the areas of activity of those associated with the initial grant-in-aid activities were as follows:

Dr. Alf S. Alving, University of Chicago, clinical investigations.
Dr. Kenneth N. Campbell, University of Notre Dame, chemical synthesis.
Dr. Nathan L. Drake, University of Maryland, chemical synthesis.
Dr. Robert C. Elderfield, Columbia University, chemical synthesis.
Dr. Joseph B. Koepfli, California Institute of Technology, chemical synthesis.
Dr. Robert E. Lutz, University of Virginia, chemical synthesis.
Dr. L. H. Schmidt, Christ Hospital Institute of Medical Research, pharmacology and parasitology.

This group together with parasitologists and clinical investigators in the Section on Chemotherapy, Laboratory of Tropical Diseases, National Institutes of

[2] Drs. George A. Carden, G. Robert Coatney (Executive Secretary), L. T. Coggeshall, A. C. Cope, Robert C. Elderfield, Clay G. Huff, R. F. Loeb, E. K. Marshall, Jr., E. A. Martin, Paul F. Russell, Capt. J. J. Sapero, Dr. James A. Shannon (Chairman), and Col. Tom Whayne.

Health, under the direction of Dr. G. Robert Coatney, agreed to coordinate their activities in order to achieve specified objectives. Under the sponsorship of the Malaria Study Section, they organized a group known as Investigators in Malaria Chemotherapy[3], which held its first meeting on October 7, 1946, and met at frequent intervals thereafter through 1948 for the purposes of assessing progress and planning new activities.

As work progressed it became evident that additional facilities were needed for chemical synthesis and parasitological evaluations. Consequently the following individuals were invited to join the cooperative group:

Dr. E. R. Becker, Iowa State College, parasitology.

Dr. A. H. Blatt, Queens University, chemical synthesis.

Dr. Wendell Gingrich, University of Texas, parasitology.

Drs. Byron Riegel and Robert Baker, Northwestern University, chemical synthesis.

Dr. Walter M. Lauer, University of Minnesota, chemical synthesis.

It was also deemed essential to undertake assessments of drug action in the field. To implement this activity, a unit was set up in Guatemala through a cooperative arrangement between that government, the Public Health Service, and the Pan American Sanitary Bureau. This field program, under the general supervision of Dr. Coatney, was inaugurated in mid-1948 and continued through June, 1950.

The activities of the Investigators in Malaria Chemotherapy can be compartmentalized roughly into studies designed (a) to evaluate previously known but inadequately assessed suppressive and therapeutic agents and (b) to develop new curative drugs.

The studies on the naphthoquinones were among the first activities carried out by the Investigator group. These compounds were of recurring interest during the OSRD malaria program both because of their unique chemical structure (the only known compounds of C, H, and O possessing significant antimalarial activity) and because they possessed prophylactic and curative activity against certain species of avian malaria. (Uncertainties with respect to chemical structure and inadequate supply of the more active compounds had made it impossible to evaluate definitively the usefulness of these drugs against human malaria during the OSRD program.) In order to obtain evaluation against mammalian malaria, the derivative which had shown the greatest promise in the wartime studies, SN-12320, was synthesized in quantities sufficient to permit pharmacological studies and evaluations against sporozoite- and trophozoite-induced *cynomolgi* malaria in the monkey and sporozoite-induced *vivax* malaria in man. These studies revealed no evidence of prophylactic or curative activities and only a low level of therapeutic effectiveness against the above infections. As a result, further work with this group of compounds was abandoned.

The 4-quinolinemethanols were another group of compounds which had shown

[3] The original membership consisted of: Drs. Alf S. Alving, Kenneth N. Campbell, G. Robert Coatney (Executive Secretary), L. T. Coggeshall, W. Clark Cooper, Nathan L. Drake, Robert C. Elderfield, Wendell Gingrich, Edward S. Josephson, R. E. Lutz, E. Mosettig, and L. H. Schmidt (Chairman).

promise in the preliminary studies of the OSRD program but had not been evaluated adequately in man. Four members of this chemical series which had shown the highest activity against avian malarias were synthesized in sufficient amounts to permit pharmacological studies in lower animals and assessment of antimalarial activity in man. The clinical studies showed that three of the compounds possessed no significant advantages over quinine in the treatment of *vivax* malaria. The fourth compound, due to its retention in the animal body, produced phenomenal lengthening of the relapse interval. However, this quality was associated with the development of photosensitivity, thus nullifying the potential usefulness of the drug. This demonstration ended active interest in these drugs.

The work of the Investigator group on the 4-aminoquinolines embodied studies on the long-term suppressive activity of chloroquine, as well as its effectiveness in single oral doses and when given intramuscularly against acute attacks of *vivax* malaria. The short-term suppressive and therapeutic activities of amodiaquin were also studied. These studies demonstrated that each of these drugs possessed high therapeutic and suppressive activity of a similar order. The effectiveness and tolerability of intramuscular chloroquine and the effectiveness of single oral doses were demonstrated clearly.

The biguanide chlorguanide which had shown considerable promise in the preliminary clinical evaluations by British, Australian, and OSRD investigators was subjected to intensive study by the pharmacologists and clinicians in the Investigator group. Extensive investigations of the pharmacology and metabolic fate of chlorguanide were carried out in experimental animals. Therapeutic studies included long-term suppressive trials and treatment of acute attacks of *vivax* malaria. Investigations of the occurrence of chlorguanide resistance were made in avian, simian, and human malarias.

The safety of long-term administration of chlorguanide was established in the pharmacological studies. It was also found that chlorguanide was degraded extensively to products which exhibited no more and sometimes less activity than the parent drug against simian malaria. Although, as shown by the British investigators, a compound was formed which was more active than the parent compound against avian malaria, subsequent work by other British investigators showed that this metabolic product was no more active than the parent drug against *falciparum* infections.

The therapeutic and suppressive studies demonstrated that chlorguanide had high activity against infections with *Plasmodium vivax*. However, the action of this drug in reducing parasitemia and fever was definitely slower than that of chloroquine. Further work showed that each of the species of avian, simian, and human plasmodia studied, acquired a high order of resistance when exposed to suboptimal doses of the drug. It was further demonstrated that the resistant characteristic of the simian plasmodium could be transmitted unaltered through the mosquito. Furthermore, when produced in sporozoite-induced infections, resistance was a property of the erythrocytic parasites only. Infections with chlorguanide-resistant strains responded normally to drugs such as quinine, quinacrine, and the 4-aminoquinolines.

The field facilities developed in Guatemala were used for a systematic com-

parison of the suppressive activities of chloroquine and chlorguanide. Although unsettled local conditions made it necessary to terminate this work prematurely, the results obtained demonstrated clearly that each of the drugs reduced the incidence of parasite carriers significantly; the greatest reduction occurred with chloroquine.

The results of these studies on the 4-aminoquinolines and chlorguanide, supplemented by observations made outside the U. S. A., established the therapeutic potentialities of both chloroquine and amodiaquin, and demonstrated that either of these drugs was superior to chlorguanide in the general management of human malaria.

The major new effort of the Investigators in Malaria Chemotherapy was directed toward development of a drug of general usefulness which would cure *vivax* malaria. This effort, centered around work on the 8-aminoquinolines during the 1946 to 1947 period, followed the pattern of the last year of the OSRD program. This involved synthesis of various congeners of pamaquine, examination of the toxicity of these compounds in monkeys, and subsequent testing of compounds devoid of neuronal toxicity against *vivax* infections in human volunteers. In addition, efforts were made to define the activity of pentaquine more precisely and to determine the best means of achieving curative results with this drug.

In 1948, the pattern of work was changed as the result of the demonstration by Dr. Schmidt and his colleagues at The Christ Hospital Institute of Medical Research that infections with *P. cynomolgi* in the rhesus monkey were the biological and chemotherapeutic counterparts of human infections with Southwest Pacific strains of *P. vivax*. The parallelism in response of the simian and human infections to known antimalarials was essentially complete both in qualitative and quantitative aspects of drug activity. This demonstration made it possible for the first time to carry out toxicological and particularly therapeutic studies in an experimental animal where it was reasonable to expect that the results could be translated to treatment of human malaria. This implemented highly controlled and quantitative assays of the activities of various drugs against the different stages of plasmodial infections, explorations of dose-therapeutic-effect relationships, and development of realistic therapeutic indices. These developments had significant effects on the intensive search for more effective and better tolerated 8-aminoquinoline derivatives and on the ultimate definition of the sphere of usefulness of the more promising of these drugs.

In the search for more effective curative drugs, collaborating chemical groups at Columbia University, University of Maryland, and University of Notre Dame synthesized 42 new 8-aminoquinoline derivatives and remade older analogs in quantities sufficient for simian and human studies. Toxicological and curative therapeutic studies were carried out on all of these compounds in monkeys at The Christ Hospital Institute of Medical Research. On the basis of these studies, 18 of the drugs were selected for human trials to be carried out at the University of Chicago. In both the simian and human investigations, four compounds with properties superior to those of pamaquine emerged. These included pentaquine

and isopentaquine, which had been developed in 1946 and 1947, respectively, primaquine[4], and SN-3883. The latter compounds, with terminal primary amino groups on the side chain, had been prepared during the OSRD program but were subjected to human study only after investigations at The Christ Hospital Institute of Medical Research had focused attention on the high tolerability of this class of compounds and their unusual activity against the early and late exo-erythrocytic stages of *P. cynomolgi*.

Whereas considerable effort was devoted during the 1948 to 1950 interval to the search for new and improved compounds, much attention was given to defining the activities and limitations of what were considered to be the more promising drugs, as well as to mechanisms of enhancing the effectiveness of known agents. Investigators at The Christ Hospital Institute of Medical Research and the University of Chicago shared in this endeavor and freely communicated their findings to one another. Two findings made initially at The Christ Hospital Institute of Medical Research and later confirmed in principle in the human subject by investigators at the University of Chicago proved to have considerable practical significance. These were (1) that quinine had no specific enhancing effect on the curative activities of 8-aminoquinolines such as pentaquine, isopentaquine, and primaquine, and (2) that these drugs could cure and in some cases prevent malaria infections when administered alone. It followed that the sole contribution of quinine was the control of erythrocytic infection. This contribution could be assumed by chloroquine or any other schizonticidal drug, or was unnecessary if the 8-aminoquinoline were administered in established infections in the interval between relapses. As will be apparent from later comments, this finding formed the basis for the "interim primaquine" regimens which allegedly have been responsible for the reduced incidence of malaria among the 1952–1953 returnees from Korea.

Despite the developments referred to above, it was generally felt by 1950 that the search for new and more effective curative drugs had reached a point of decreasing productivity. Recognizing this, the Tropical Medicine Study Section[5] of the Public Health Service appointed an ad hoc Committee[6] for the purpose of appraising the current status of malaria therapy and outlining areas which would require special attention in future studies. After a thorough review of postwar developments in this field on November 1, 1950, this Committee recommended that in the absence of new leads, synthesis and lower animal testing of additional 8-aminoquinolines be discontinued so that all clinical facilities avail-

[4] A practical method of producing primaquine was worked out at Columbia University during 1949–1950 and was successfully transferred to full-scale manufacturing operation through the cooperation of Abbott Laboratories, Eli Lilly and Company, Parke-Davis & Company, and Winthrop Stearns Inc. This development was particularly fortunate in view of the later demand for primaquine for the interim treatment of *vivax* malaria.

[5] This Study Section resulted from the consolidation (1949) of the Malaria Study Section and the Tropical Disease Study Section.

[6] Dr. G. Robert Coatney, Col. Wesley C. Cox, Drs. E. K. Marshall, Jr., Harry Most, Major John Rizzolo, Dr. Paul F. Russell (ex officio), and Mr. Francis L. Schmehl (ex officio), Dr. Leon H. Schmidt (Chairman).

able could be utilized in defining precisely the therapeutic status of pentaquine, isopentaquine, primaquine, and SN-3883. This recommendation, although opposed by some members of the Investigator group, was nevertheless affirmed by the Tropical Medicine Study Section. This decision marked the end of the large-scale cooperative work and the first phase of postwar research in malaria chemotherapy.

Before passing to the second phase of postwar research in malaria, it is pertinent to note that studies carried out incidental to the main work on chemotherapy produced significant findings on the biology of the disease. Among these were contributions to knowledge of the natural history of untreated simian and human malarias, development of immunity, the exoerythrocytic cycle of simian and human malarias, and information on the role of the spleen in regulating the pathogenicity of malaria parasites in monkeys. In addition, major contributions were made to basic knowledge in pharmacology, some of which were later applied to work on the chemotherapy of leukemia and poliomyelitis.

Shortly after the decision of the Tropical Medicine Study Section, the appearance of *vivax* malaria in Service personnel returning from Korea turned the interests of our Armed Services to the problem of curative malaria therapy. At the suggestion of the Services, an *ad hoc* Committee[7] was brought together at the National Research Council on December 29, 1950, for the purpose of appraising the malaria therapy problem. After full discussion, this group concluded that chloroquine was entirely adequate for suppression and treatment of acute infections caused by *P. vivax* and for suppression, treatment, and cure of infections with *P. falciparum*. There was need, however, for a generally useful drug for the radical cure of *vivax* infections. It was felt that one of the more promising 8-aminoquinolines developed during the postwar program might satisfy this need. The problem of prime importance was to define the relative merits of these drugs and the most effective regimen for their administration. This group of conferees concluded, as had the Tropical Diseases Study Section earlier, that the compounds of greatest promise were pentaquine, isopentaquine, primaquine, and SN-3883.

The group of investigators headed by Dr. Alving at the University of Chicago was charged with responsibility for a portion of the evaluation of these drugs in curing established *vivax* infections in human volunteers. In order to insure complete evaluation of these drugs in a short time, it was considered necessary to establish a second clinical testing facility. At the suggestion of the National Research Council, this second facility[8] was established at the U. S. Penitentiary,

[7] Drs. Alf S. Alving, Kenneth Blanchard, G. Robert Coatney, Arthur C. Cope, Robert C. Elderfield, Robert F. Loeb (Chairman), E. K. Marshall, Jr., James A. Shannon, Leon H. Schmidt. This group, and Dr. L. T. Coggeshall, became the Subcommittee on Malaria of the NRC on October 12, 1951. This subcommittee was replaced by the Panel on Malaria (Drs. G. Robert Coatney, Leon H. Schmidt, and James A. Shannon (Chairman)) on November 4, 1953.

[8] Facilities for human testing had been discontinued by the Public Health Service on 30 June 1950.

Atlanta, Georgia, under Public Health Service-Department of the Army auspices. Work at this installation, under the direction of Dr. Coatney, was begun in April, 1951.

Before evaluations could be completed at either of these installations, the urgency created by the appearance of numerous cases of acute malaria among returnees from Korea made it necessary to improvise measures for the cure of this infection. At a meeting on July 3, 1951, called by the Surgeon General, Department of the Army, it was decided to focus attention on primaquine, specifically to investigate (a) the effectiveness of this compound in eradicating established infections in Korean returnees and (b) the feasibility of administering the drug at a dosage of 15 mg. daily for 14 days to all veterans returning from Korea via ship. In the absence of extensive toxicological data it was deemed necessary to study the tolerability of the drug in human volunteers before inaugurating the treatment program. This toxicological investigation, involving 1,000 Army personnel, was carried out at Fort Benning, Georgia, and Fort Knox, Kentucky[9]. This test demonstrated the over-all safety of administering a single dose of 15 mg. primaquine daily to military personnel on full duty. Following this demonstration a program for administering primaquine aboard ship was evolved. The first trial of this procedure was made on a group of veterans returning to the United States on September 18, 1951. Administrative difficulties arising during this and a subsequent trial made it impossible to appraise the therapeutic effectiveness of this regimen. The safety of the regimen was clearly evident, however, and the administration of 15 mg. daily for 14 days was adopted as a general procedure pending more precise data from the highly controlled studies on prisoner volunteers and the curative evaluation tests under way at continental military installations.

Collateral studies at the University of Chicago on prisoner volunteers had demonstrated that primaquine at 30 mg./day was tolerated by Caucasians; in Negroes, however, such doses evoked intravascular hemolysis in 5 per cent of the subjects.

The data from controlled studies on prisoner volunteers, available in January, 1952, demonstrated the superiority of primaquine over pentaquine, isopentaquine, and SN-3883 as a curative drug. The results obtained against active *vivax* infections among returnees from Korea showed the high order of curative effectiveness of primaquine and its superiority over pamaquine. Studies of the effectiveness of interim treatment with primaquine on selected returnees demonstrated the curative effectiveness of such a procedure. This composite information, together with toxicological data obtained on prisoner volunteers provided a solid basis for the adoption of interim treatment with primaquine as a measure for the cure of Korean *vivax* malaria. This procedure was eventually applied to all veterans returning from Korea via ship and has been associated with the virtual elimination of malaria among returnees from Korea.

[9] This investigation was under the guidance of Drs. Coatney and Alving, respectively. These investigators and Dr. Ralph Jones, Jr., were responsible for subsequent studies at military installations.

Late in 1950 interest was stimulated in a new type of antimalarial drug which had been produced in the Wellcome Research Laboratories, New York, as a by-product of investigations on purines and pyrimidines as carcinolytic agents. Interest centered on pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, Daraprim). Extensive studies were carried out on the pharmacology of this drug in lower animals and on its activity against avian and simian malarias. Subsequently, work was carried out on the pharmacology of the compound in man and its activity against *P. falciparum* and *P. vivax* infections. Therapeutic studies in both lower animals and man showed that the compound had higher activity against erythrocytic parasites than any known antimalarial drug. However, the drug was slower than chloroquine in controlling active simian and human infections. The compound possessed activity against the exoerythrocytic stages of all species of malaria parasites studied. In the case of *P. vivax* this activity was strong enough to effect suppressive cure in a high proportion of infections. Resistance to pyrimethamine developed rapidly during inadequate treatment of erythrocytic infections with avian, simian, and human plasmodia. In the case of *cynomolgi* and *vivax* malarias, the resistant characteristic could be transferred unchanged through the mosquito. Pharmacological studies in the simian subject demonstrated that repeated administration of pyrimethamine produced significant deleterious changes in the bone marrow, kidney, and adrenal cortex. Tolerability studies in man attested to the safety of the currently recommended doses of pyrimethamine but showed that at a sevenfold increase in dosage, changes occurred in the bone marrow similar to those exhibited by the monkey.

The capacity of pyrimethamine to function as a suppressive cure strongly recommended its use by our Armed Services. However, its potential for inducing resistant strains and its slowness of action when used in treatment of acute attacks pointed up disadvantages not possessed by chloroquine.

In conclusion, in the period from 1946 to 1954 there were marked developments in malaria chemotherapy. The position of chloroquine and amodiaquin in the suppression and treatment of *vivax* and *falciparum* malarias and in the cure of *falciparum* infections was established firmly. The usefulness and limitations of chlorguanide were defined with reasonable certainty. A generally useful drug for the cure of relapsing *vivax* malaria was developed in primaquine, and the value of this agent was proved in both laboratory and field investigations. Finally, a new compound, pyrimethamine, possessing interesting possibilities as a suppressive antimalarial drug was introduced recently. The ultimate significance of these developments for world-wide control of malaria remains to be appraised. It seems likely, however, that satisfactory measures are now at hand for suppression, treatment, and cure of all human malarias. This remarkably favorable position of malaria therapy is in marked contrast to the uncertainty of pre-World War II. It is a tribute to all who took part in the OSRD and postwar malaria researches.

TOXICITY STUDIES OF PYRIMETHAMINE (DARAPRIM[1])

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Because of the limited information available as to the toxicity of pyrimethamine (Daraprim), 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine, when studied under conditions of close laboratory supervision, the Subcommittee on Malaria, National Research Council requested this investigation. The work was begun in November, 1952 and was carried on simultaneously with studies by the United States Public Health Service (Myatt, Coatney, Hernandez, and Burton, 1953). In addition to general observations of toxicity, particular emphasis was placed on bone marrow findings when 25 milligrams of pyrimethamine were administered weekly for 6 months to a series of volunteers.

VOLUNTEERS

A total of 214 men have been given pyrimethamine on various schedules. Forty-one Caucasians and 40 Negroes participated in toxicity studies and 133 Caucasians were given the drug as malaria prophylaxis or therapy.

CLINICAL INVESTIGATIONS

A. *Effect of pyrimethamine on healthy men*

1. *Effect on peripheral blood, urine, and body weight.* Eighty-one volunteers were given 25 mg. of pyrimethamine weekly for 6 months. No significant changes were observed in hemoglobin, white blood count or urine, all of which were done every 2 weeks during administration of the drug. In 38 of these volunteers, studies of renal function (phenolsulfonphthalein excretion) were also performed before and every 4 weeks during administration of pyrimethamine. None of the subjects exhibited a depression of renal function as determined by this method. Forty-six of the volunteers were weighed before and at intervals during administration of pyrimethamine. No significant change of weight occurred during the observation period of 6 months.

2. *Effect on bone marrow.* One hundred and thirty sternal bone marrow aspirations were performed on 45 volunteers receiving 25 mg. of pyrimethamine weekly. The men were divided into 4 groups, each composed of half Negro and half Caucasian. The first group was composed of 9 men whose bone marrow was examined initially, after 9 weeks, and again after 15 weeks of drug. This group received, in addition to pyrimethamine, 1.0 gm. of sulfadiazine daily for the last

[1] This work was supported (in part) by the Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-20 with the Department of Medicine, University of Chicago, Chicago, Illinois.

The investigations reported in this paper would not have been possible except for the cooperation of the inmates and the administrative staff of Stateville Penitentiary.

6 weeks of the study. The second group was composed of 8 men, whose bone marrow was examined initially, after 9 weeks, after 15 weeks, and again after 26 weeks of drug. The third group of 22 men had marrow examinations performed initially, and after 26 weeks of drug. A fourth group of 6 men received initial, 26-week and 28-week marrow examinations. This group received 4.0 gms. sulfadiazine daily in addition to pyrimethamine for the last 2 weeks of the study. Smears and imprint (touch) preparations were made from all marrow specimens and fragments of marrow were fixed for sectioning. All smears and imprints and some of the sectioned fragments were examined microscopically.

No specific abnormality was found in any of the specimens. Megaloblastic transformation of the marrow was not observed in any of the specimens studied in this series.

3. *Dermatoses.* Two Negro subjects receiving 25 mg. of pyrimethamine weekly developed identical skin eruptions consisting of discrete maculopapules, some of which progressed to central necrosis and subsequently healed with scar formation. The rash developed after 2 to 3 months of drug administration. Skin biopsies showed exudative dermatitis. There were prominent perivascular inflammatory changes in the upper corium. Re-administration of 25 gm. of pyrimethamine weekly for 11 weeks to both subjects did not result in recurrence of these manifestations.

B. *Effect of pyrimethamine on men with malaria*

1. *Anemia.* One Caucasian who received pyrimethamine therapy for *falciparum* malaria developed severe anemia and leukopenia. The subject had been inoculated intravenously with trophozoites of a "pyrimethamine-resistant" strain of *P. falciparum*. He received 3 courses of therapy consisting of 25 mg. of pyrimethamine daily for 7 days. The remissions were of short duration and had required new courses of drug after intervals of 8 to 9 days. During the third course of therapy, marked pallor was observed and the hemoglobin was found to be 8.0 gms. per cent. The malaria was treated with chloroquine on the 38th day of the experiment. A bone marrow examination was done on day 40. Sections and smears of the sternal marrow revealed an increased number of young cells of the granulocytic series, eosinophilia, and plasmocytosis in a highly cellular specimen. Most of the red cell precursors were megaloblasts indistinguishable from those of pernicious anemia. However, the peripheral blood picture was distinctly that of a microcytic, hypochromic anemia. No specific therapy was given. Recovery was spontaneous but slow. A second bone marrow examination on day 137 was erythroblastic. A gastric analysis at this time showed no free acid in fasting gastric contents, but after histamine, free acid was present. Further hematologic investigations established that the patient had thallasemia minor. He was given another course of *falciparum* malaria without pyrimethamine therapy. After 14 days his hemoglobin had dropped from 13.0 to 8.6 gms. per cent, but the sternal marrow was *erythroblastic*. After treatment with chloroquine, complete hematological recovery occurred within 6 weeks. The same schedule of pyrimethamine

therapy as had been used initially was then repeated without inoculating the patient with malaria. The patient tolerated this schedule without development of anemia or leukopenia.

2. *Purpura.* One subject, a Caucasian, developed numerous petechial hemorrhages on the lower extremities during pyrimethamine therapy of a relapse of *vivax* malaria. Previous treatment (two 8-week courses of pyrimethamine at 25 mg. weekly) had been given without incident. During therapy of the second relapse with 75 mg. pyrimethamine on the first day, and 25 mg. daily for the next 2 days, a purpuric rash was noted on the lower extremities. Blood counts were normal except for a platelet count of 66,000. The sternal marrow showed a borderline decrease in the number of megakaryocytes and moderate granulocytic hyperplasia with a slight left shift. The petechiae cleared and the platelet count returned to normal in less than 2 weeks. Re-administration of pyrimethamine in the absence of malaria (25 mg. daily for 10 days) failed to affect the platelet count or to cause purpura.

3. *Dermatoses.* One Caucasian subject who received 75 mg. of pyrimethamine on the first day and 25 mg. on each of the next 2 days for treatment of vivax malaria developed a diffuse pruritic rash consisting of confluent rose-colored macules with fine superficial scaling. This cleared in 4 days. Re-administration of 25 mg. of pyrimethamine daily for 14 days seven weeks later failed to cause any cutaneous manifestations.

DISCUSSION

No significant toxic effects were noted when 25 mg. of pyrimethamine were administered weekly to 81 men for 6 months. These findings are in agreement with those reported by Myatt, Coatney, Hernandez, and Burton (1953).

Studies by Myatt, Hernandez, and Coatney (1953) demonstrated that 25 mg. of pyrimethamine daily for 49 days induced a megaloblastic anemia in one-half of a group of volunteers. In our series of cases, one man with thallasemia minor and *falciparum* malaria developed a megaloblastic bone marrow when given pyrimethamine on a schedule of 25 mg. daily. Administration of pyrimethamine alone failed to cause any change in the blood counts of this man and the anemia which developed with another two-week course of *falciparum* malaria was not accompanied by megaloblastic transformation of the marrow. Megaloblastic transformation therefore occurred in a patient receiving pyrimethamine, only when the excessive erythropoietic demands of both the hemolytic states, thallasemia minor and *falciparum* malaria, were made on the marrow.

It appears doubtful that pyrimethamine therapy played a role in the one case of purpura and thrombocytopenia that was observed, since these conditions are known to occur in untreated malaria (Shrager and Kean, 1946; Diggs, 1944), and since a previous course and re-administration of pyrimethamine to the same patient in the absence of malaria failed to cause either thrombocytopenia or purpura.

Similar failure to reproduce any of the skin lesions occurring during adminis-

tration of pyrimethamine suggests, although it does not establish, that the dermatoses observed were incidental findings not related to the administration of pyrimethamine.

SUMMARY

The administration of pyrimethamine (Daraprim) to 81 men in doses of 25 mg. weekly for 6 months failed to produce toxic effects. In 133 other men, who received the drug as malaria therapy on various schedules, no toxic effects which could be unequivocably attributed to pyrimethamine were observed.

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THE TREATMENT OF MALARIA WITH HYDROXYCHLOROQUINE

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The antimalarial drug, hydroxychloroquine (Plaquenil®, Winthrop-Stearns, Inc.) has the formula 7-chloro-4-[4-(N-ethyl-N-β-hydroxyethylamino)-1-methylbutyl-amino] quinoline diphosphate. Its synthesis was reported by Surrey and Hammer in 1950 and its physiologic and pharmacologic properties were elucidated by Dennis *et al.* (1952) and McChesney and associates (1954). These experimental studies indicated the properties of hydroxychloroquine to be similar to those of other 4-aminoquinoline drugs such as chloroquine and amodiaquin.

The several reported clinical studies were all made in Latin America. Loughlin *et al.* (1952) were favorably impressed by their observations on 75 *P. falciparum* infections treated in Haiti with single oral doses ranging from 1.0 gram for young children to 2.0 grams for adults. Garzo Brito (1952) and his associates in Mexico City draw optimistic conclusions from the results of treatment of 91 patients on six dosage schedules. Clark (1954) has reported good results in malaria suppression in a rural Panamanian village, employing a weekly treatment with tablets containing 30 mg. primaquine and 300 mg. hydroxychloroquine. In Honduras, we (Hoekenga, 1953) achieved encouraging preliminary results in 68 acute malaria infections (21 with *P. falciparum*, 47 with *P. vivax*) treated with a single oral dose of 1.25 grams of the drug, and in the treatment of 14 acute *P. falciparum* infections with an intravenous dose of 0.36 gram in 500 cubic centimeters of normal saline. In a more recent publication (Hoekenga, 1954), the number of orally treated infections in this series had increased to 125, and the same good results were obtained. In none of these clinical reports has there been any suggestion of drug toxicity except for transient vertigo in one of Loughlin's patients.

It is the purpose of this paper to bring up to date the results of 213 acute malarial infections treated with hydroxychloroquine—150 orally, 37 intramuscularly, and 26 intravenously.

MATERIAL AND METHODS

All patients included in this study were Honduran mestizos, representing various mixtures of Indian, Negro and Spanish ancestry. The ages ranged from seven years to sixty-five years. Seventy-eight per cent of the patients were males and 22 per cent were females. All had acquired their malaria in northwestern Honduras and were hospitalized at the Tela Railroad Company (United Fruit Company) hospital located at La Lima. In this area, *Anopheles albimanus* is the principal vector. Although there is some seasonal variation, malaria is endemic all year; because of this endemicity, the adult native population may be considered to have acquired a partial immunity to local plasmodia strains.

A single oral dose of 1.25 grams was given to 75 patients with *P. vivax* infections and 50 patients with *P. falciparum*. These are the same patients referred

to above (Hoekenga, 1954); they are included in this paper for completeness and for comparison with the other schedules. A smaller single oral dose of 0.75 gram was given to 11 *P. vivax* and 14 *P. falciparum* infections. The dose given to all of the 63 parenterally treated cases of *P. falciparum* malaria was 360 mg. For the 26 patients in the intravenous group, the drug was included in 500 cc. of normal saline, or 5 per cent glucosaline solution. For the 37 patients in the intramuscular group, this amount was included in 9.0 cc. of normal saline solution, half being injected into each gluteal area.

Oral temperature on all patients was recorded every four hours. Thick blood films were examined every 6 hours. The amounts of drug and the time of administration were not related to parasite density, to the stage of parasite cycle observed in the blood film, or to the degree of fever.

RESULTS

Immediate results. With the oral, intravenous and intramuscular regimens there was usually rapid clinical improvement. Symptoms caused by malaria disappeared in 18 to 48 hours. The time required for disappearance of fever and parasitemia on the various regimens is detailed in Table 1. Of the orally treated patients, there were four immediate failures (16 per cent) among the 0.75 gram group, and three immediate failures (2.4 per cent) among the 1.25 gram group, indicating the higher dosage to be more effective. There were no immediate treatment failures among the intravenously treated patients and only one (2.7 per cent) among the intramuscularly treated ones (Table 1).

Late results. A 20 year old girl with *P. falciparum* infection, who had responded very favorably to the intravenous dosage, was rehospitalized with clinical and parasitological relapse after a three weeks' interval. Two patients with *P. vivax*

TABLE 1

Results of treatment of acute attacks with single doses of hydrozychloroquine

	No. of Cases	Average Number of Hours* Until:		Immediate Failures	
		Temp. remained normal	Parasites disappeared	Number	Per cent
Single oral dose of 0.75 gram					
P. falciparum	14	26.2	38.2	2	14.3
P. vivax	11	28.3	33.5	2	18.2
Single oral dose of 1.25 grams					
P. falciparum	50	20.9	30.3	2	4.0
P. vivax	75	18.7	24.0	1	1.3
Single intramuscular dose of 0.36 gram (*P. falciparum* only)	37	21.7	33.0	1	2.7
Single intravenous dose of 0.36 gram (*P. falciparum* only)	26	24.3	21.5	0	0.0
Total cases	213	—	—	8	3.7

* The tabulation of the number of hours required for normal temperature and for parasite disappearance does not include the immediate treatment failures.

malaria on the 0.75 gram schedule relapsed after 6 weeks. These three patients were re-treated with 1.25 gram orally, and none relapsed again during one year's observation.

Toxicity. Frequent clinical observations and a battery of laboratory tests were employed in an effort to detect toxic effects. The latter included hemoglobin determinations, red blood cell counts, white blood cell counts, urinalyses, serum bilirubin estimations and determinations of bromsulphalein clearance and Hanger cephalin-cholesterol flocculation. No toxic effects were noted with any of the treatment regimens, neither visual disturbances, gastro-intestinal symptoms, evidence of liver or kidney damage nor effect on the hemopoietic system. In none of the 37 individuals who received intramuscular hydroxychloroquine was there any local reaction to the gluteal injections. In none of the 26 patients who received intravenous medication was there appreciable change in the pulse or in the arterial blood pressure. No adverse effects were noted on patients with pregnancy, severe malnutrition or severe anemia.

SUMMARY

Two hundred and thirteen Hondurans were treated for acute malaria with hydroxychloroquine or Plaquenil® (7-chloro-4-[4-(N-ethyl-N-β-hydroxyethyl-amino)-1-methylbutyl-amino] quinoline diphosphate). Twenty-five of them received a single oral dose of 0.75 gram and 125 received 1.25 grams. Twenty-six others with *P. falciparum* infections were given an intravenous dose of 0.36 gram in 500 cc. of normal saline, and 37 with *P. falciparum* infections were given 0.36 gram intramuscularly in 9.0 cc. of saline. Except with the lower oral dose, both the immediate and late results were quite good—comparing favorably with the other 4-aminoquinolines in common use. Toxic side effects were not noted on any of the regimens.

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ANTIAMEBIC ACTION OF 5-CHLORO-7-DIETHYLAMINOMETHYL-8-QUINOLINOL AND OF OTHER SUBSTITUTED 8-QUINOLINOLS IN VITRO AND IN EXPERIMENTAL ANIMALS

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Until more is known concerning the physiology and pathogenesis of *Endamoeba histolytica*, the search for new antiamebic drugs probably will consist mainly of empirically testing as many different substances as possible and of systematically examining chemical series that have already yielded useful drugs. This report, stemming from the latter approach, represents part of a continuing effort to find new drugs among the substituted 8-quinolinols. Further exploration of this series is encouraged by the clinical use of 5,7-diiodo-8-quinolinol (diiodohydroxyquinoline, Diodoquin), 5-chloro-7-iodo-8-quinolinol (iodochlorohydroxyquinoline, Vioform) and sodium 7-iodo-8-quinolinol-5-sulfonate (chiniofon) for intestinal amebiasis.

MATERIALS AND METHODS

In vitro tests. Except for specified variations in the culture media, all drugs were examined under the standardized conditions used in previous work (Thompson *et al.*, 1950). The University of Chicago strain of *Endamoeba histolytica*, cultured with a mixture of bacteria, was used. Stock cultures were grown in LEL medium (a diphasic preparation of whole egg and Locke's solution, Reardon and Rees, 1939) supplemented with rice starch. To avoid complete amebicidal action if the drug acted only upon the bacteria of the culture, a massive microbial inoculum, a 24-hour period of exposure to the drug, and incubation in an atmosphere of CO_2 were used. High concentrations of various potent antibacterial drugs, such as penicillin and streptomycin, alone or combined, were not completely amebicidal under these conditions (Thompson *et al.*, 1950).

Each drug was tested in an essentially protein-free, buffered egg yolk infusion of the Balamuth (1946) type (Difco), usually supplemented with 0.5 per cent liver extract. Approximately one-fourth of the drugs were tested in egg yolk infusion without liver extract. The type of medium used in each instance is indicated in the tables. The average increase in the number of untreated amebae during the test period in these media was approximately 75 per cent. The protein-deficient medium provided a sensitive test for drugs that might be subject to protein binding.

Approximately two-thirds of the compounds were tested also in the presence of 4.3 per cent gastric mucin (Wilson, 1701-W) in egg yolk infusion or, rarely, in the overlay of LEL medium. Nitrogen determinations indicated that this concentration of mucin contained approximately 1.75 per cent protein, which is roughly equivalent to the concentration of 20 per cent horse serum.[1]

[1] Kindly performed by Dr. Gertrude Rodney of these laboratories.

Mucin consistently stimulated the growth of the amebae in egg yolk infusion, giving an average 300 to 400 per cent increase in control cultures during the test period rather than an approximate 75 per cent increase. The cultivation of *E. histolytica* in the presence of gastric mucin has been described also by Ratcliffe (1934). The mucin medium was selected for information on the effects of a natural proteinaceous substance of the alimentary tract on antiamebic action; it was hoped that this information would not only be useful in selecting drugs for trial in intestinal amebiasis but would also favor correlation between activity *in vitro* and *in vivo*.

Drugs were dissolved when possible with the aid of 0.1 N NaOH, 0.1 N HCl, or organic solvents in 0.2–0.5 ml. of the menstruum; this was made up to 2 ml. with egg yolk infusion, and the pH was adjusted to approximately 7.0. The stock preparations then were diluted with egg yolk infusion to yield the desired concentration when 2 ml. of drug preparation were added to 2.5 ml. of medium, and the amebic inoculum (including rice starch) was added in a volume of 0.5 ml. Solvent controls were included to rule out nonspecific effects for drugs dissolved in organic solvents. Amebicidal action was concluded if no viable amebae were detected by direct microscopic examination. Amebae of questionable viability were examined as wet-mount preparations in a solution of eosine in physiologic saline. Experience with amebae killed by heat and otherwise had shown that living trophozoites fail to stain with eosine while dead organisms take up the stain readily. Drugs usually were tested at 2-fold dilution increments, and amebicidal endpoints were considered as midway between the observed effective and next lower concentration. Some were tested only at 10-fold dilution increments. In these cases the assigned endpoint is accompanied by the appropriate value to indicate the probable range of amebicidal concentration.

The F-22 strain of *E. histolytica* cultured in association with the Culbertson strain of *Trypanosoma cruzi* rather than bacteria, also was used. Test methods were similar to those of Nakamura and Anderson (1951). Preparations consisted of 3 ml. of *T. cruzi* culture in Anderson's (1951) modification of Phillip's (1951) medium, 0.75 ml. of 0.3 per cent sodium thioglycollate solution in physiologic saline, 0.75 ml. of sediment from stock ameba-cruzi cultures, and 0.5 ml. of drug solution in physiologic saline. The final preparations contained 1800 amebae/ml. and were sealed with petrolatum in 15 ml. centrifuge tubes. Drugs were tested at 2-fold dilution increments for 48 hours at 37.5°C. Effects were determined by searching for amebae through the sides of the warm tubes and in the sediment with the aid of eosine.

In vivo tests. Drug dosages in animals are expressed in terms of the free base. A standardized procedure for testing drugs against intestinal amebiasis in rats has been described elsewhere (Thompson *et al.*, 1950). Animals ranging from 19 to 22 days of age and of either sex, were inoculated intracecally with 100,000 trophozoites of *E. histolytica* (strain 200, BNIH), cultured with a mixture of bacteria in LEL or Cleveland-Collier medium (Difco) plus rice starch. Drugs were administered in the diet from approximately 3 hours after infection until autopsy 7 days later. Therapeutic effects are calculated from treated and parallel

untreated groups, and are expressed as the per cent reduction in the average degree of infection (ADI) and per cent animals cleared (see footnotes "d" & "e", Table 1). The ADI is based on the amount of cecal pathology and the density of amebae found in the cecal mucosa. An active compound is arbitrarily considered to be one which suppresses the ADI in rats 50 per cent or more, and/or clears 50 per cent or more of the animals at levels below the MTD. For the sake of brevity, data on untreated animals are not shown for the individual compounds. Untreated controls, usually 10, were included in each experiment; among 1410 such animals in 137 experiments, the mean incidence of infection was 92.6 per cent (range 36 to 100) and the mean ADI was 3.3 (range 1.4–4.7). Growth and mortality comparisons between treated and untreated groups are indicative of drug tolerance or acceptance. The tolerance was considered good when treated groups gained at least 60 per cent as much weight as controls, growth suppression when they gained only 20–60 per cent, maximum tolerated dose when they gained less than 20 per cent, and lethal when most of them died. The drug intake at a given diet concentration is also indicative of the degree of tolerance or acceptance. An unrestricted intake is proportional to the diet concentration. Based on normal food consumption of infected animals, a diet containing 0.5 per cent of drug would yield an intake of approximately 700 mg. of drug/kg./day.

Drugs were tested against amebic colitis in dogs as in previous work (Thompson and Lilligran, 1949; Thompson *et al.*, 1950). Dogs of either sex, purchased from the city pound, were maintained on a diet of canned salmon supplemented with brewers' yeast and cod liver oil. Infections were induced intrarectally with strain 200 *E. histolytica* procured from cultures or donor dogs. Drugs were administered in gelatin capsules for 10 days (two 5-day periods separated by an interim of two days). Colonic aspirates were examined directly for amebae three times weekly for evidence of therapeutic effect. Animals negative for amebae during 4 weeks after treatment were considered cured; when as many as 5 consecutive samples were negative, the drug was considered to have exerted a suppressive effect. These criteria are based on observations during the past 5 years of the general course of infection in some 250 dogs. Thus, spontaneous recovery has occurred in only 4 of 225 untreated dogs observed during a mean period of 24 days; 5 consecutive negative samples occurred at the rate of about only one in 20 untreated animals.

Drugs were tested against amebic hepatitis as in previous work (Thompson and Reinertson, 1951). Golden hamsters were inoculated intrahepatically with 50,000 strain 200 trophozoites. Drugs were given twice daily for 4 days. The first dose was administered approximately 3 hours before infection and the last dose about 18 hours before the animals were sacrificed to assay the effects of treatment. Autopsies were performed approximately 92 hours after infection; the hepatic lesions were excised, weighed, and examined for amebae. Therapeutic effects were based upon the suppression of lesion development and the presence or absence of amebae in the lesions.

Sources of Compounds. Most of the substances examined are known com-

pounds. The 7-mono- and dialkylaminomethyl derivatives were prepared by J. H. Burckhalter, University of Kansas, and by William H. Edgerton of these laboratories, via the Mannich reaction on the corresponding 8-quinolinols (Burckhalter and Edgerton, 1951, and Edgerton and Burckhalter, 1952). Other donors of compounds were C. A. Vanderwerf, University of Kansas (Helin and Vanderwerf, 1952); Henry Gilman, Iowa State College; J. P. Phillips, University of Louisville; Harry S. Mosher, Pennsylvania State College and Stanford University; Eastman Kodak Co., Rochester, N. Y.; Monsanto Chemical Co., St. Louis, Mo.; Martin L. Black, Horace DeWald, Leonard Doub, Patricia A. Jones, Eugene Payne, F. H. Tendick, O. L. Stevenson, Constance T. Walker, and Donald F. Walker, of these laboratories. Other compounds were purchased from regular commercial sources.

SCREENING TEST RESULTS

This phase of the study was oriented around the primary objective of searching the 8-quinolinol series for potentially useful drugs. This purpose, along with other factors such as the availability of drug samples and a lack of general promise, frequently resulted in only cursory examination of a substance. Therefore, the available data are more useful in pointing to certain relationships between structure and activity than in providing for a critical comparison of the individual compounds. Unless specified otherwise, statements in the text regarding activity *in vitro* refer to effects observed in the protein-free medium.

1. *Relationships between chemical structure and activity* in vitro *and in intestinal amebiasis*

5-Substituted 8-quinolinols. The effects of 23 5-substituted 8-quinolinols *in vitro* and in rats are tabulated in Table 1; two of these compounds were tested in dogs (Table 7). *In vitro*, 8-quinolinol (1) and 5-methyl-(2), 5-chloro-(5), 5-bromo-(6), 5-acetyl-(7), and 5-anilinosulfonyl-8-quinolinol (19) were amebicidal at <20 μg/ml.; 5-chloro-(5) and 5-anilinosulfonyl-8-quinolinol (19) were active against intestinal infections in rats. 8-Quinolinol (1) and 5-acetyl-8-quinolinol (7) possessed therapeutic action in dogs. 5-Benzoyl-(11), 5-cinnamoyl-(12), 5-carboxy-(13), 5-(1-iminoethyl)-(15), 5-amino-(16), 5-phenylcarbamoyl-(18), 5-nitroso-(20), 5-nitro-(21), and 5-(*p*-acetylaminophenylazo)-8-quinolinol (23) were amebicidal in the range of 20 to 200 μg./ml.; 5-benzoyl-(11) and 5-nitro-8-quinolinol (21) were effective in rats. The introduction of large alkyl groups (3, 4) or of large acyl groups (9, 10) at position 5 caused a sharp decrease in activity *in vitro*. Compounds 9, 18, 22 and 23 were less active in the presence of mucin.

Halogenated 8-quinolinols. In addition to the effects of 5-chloro-(5) and 5-bromo-8-quinolinol (6) discussed in the previous section, the activities of 11 halogenated compounds are summarized in Tables 2 and 7. 5-Chloro-7-iodo-(29), 5,7-dichloro-2-methyl-(26), 5,7-diiodo-8-quinolinol (30), and 7-iodo-8-quinolinol-5-sulfonic acid (31) were active *in vitro* and in experimental animals. Compounds 29, 30 and 31 represent the active components of drugs that have been used

TABLE 1

5-Substituted 8-quinolinols. Screening test results against E. histolytica *in vitro and in rats*

No.	Substituent	*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
			% in diet	Mg./Kg. daily, 7 days		Suppression of infection[d]	Cured[e]
1	H	15[a]					
2	CH_3	8[b,a]					
3	$(CH_2)_6CH_3$[m]	>2000[b] (>2000[b])					
4	$(CH_2)_{15}CH_3$	>2000[b] (>2000[b])					
5	Cl[o]	15[b]	1.0	1157	5	80	80
			0.5	519	4	77	50
			0.25	256	5	64	60
6	Br	4					
7	$COCH_3$	4					
8	$COCH_2CH_3$	110 ± 90[b,a] (110 ± 90[b])					
9	$CO(CH_2)_7CH_3$[m]	188[b]) (>2000[b]	0.25	298	5	0	0
10	$CO(CH_2)_{14}CH_3$[m]	>2000[b]) (>2000[b]					
11	COC_6H_5	110 ± 90[b,a] (110 ± 90[b])	0.47	454	4	80	75
12	$COCH{=}CHC_6H_5$	94[b]	0.5	610	5	30	40
13	COOH	188[b]	0.4	398†	5	0	0
14	SO_3H	>2000	0.5	629†	4	0	0
15	$C(=NH)CH_3$	188	0.5	655	5	33	33
16	NH_2	188					
17	$NHCOCH_3$	1100 ± 900[b,a] (1100 ± 900[b])					
18	$CONHC_6H_5$	110 ± 90[b,a] (>2000[b])					
19	$SO_2NHC_6H_5$	9[b]	1.0	1211	5	61	54
			0.5	564	4	30	25
20	NO	75	1.0	1313†	4	16	0
			0.5	622†	5	13	0
21	NO_2	38	0.92	659†	10	82	55
22	[N=N—C_6H_3—*m*—$CH_2N(C_2H_5)_2$ *p*—OH]	1100 ± 900[b,a] (>2000[b])					
23	[N=N—C_6H_4—*p*—$NHCOCH_3$]	94[b] (>2000[b,z])					

TABLE 1—*Continued*

[a] Maximum tolerated dose. Animals usually survived, but gained less than 20% as much weight as the control animals.

[aa] Lethal. Excessive deaths relative to controls coupled with virtually complete growth suppression and greatly restricted drug intake in survivors.

† Growth suppression. Animals gained only 20–60% as much weight as the control animals.

[a] All compounds were tested as solutions unless otherwise indicated. Test results without parentheses refer to effects observed in the essentially protein-free medium, which contained liver extract except when specified otherwise, see footnote "z". Test results in parentheses refer to effects in the mucin-containing medium.

[b] Tested as a suspension.

[c] Treated for only six days.

[d] (ADI Untreated minus ADI Treated/ADI Untreated) × 100. ADI represents Average Degree of Infection as defined previously (Thompson et al., 1950).

[e] (Per cent untreated infected minus per cent treated infected/Per cent untreated infected) × 100

[f] Tested under aerobic conditions.

[g] R_2N represents the 1-piperidyl group.

[h] R_2N represents the 1-pyrrolidyl group.

[i] R_2N represents the 4-morpholinyl group.

[j] R_2N represents the 4-phenyl-1-piperazyl group.

[k] Tested as the monohydrochloride.

[l] Tested as the dihydrochloride.

[m] Tested as the monohydrobromide.

[n] Tested as the dihydrobromide.

[o] Tested as the trihydrochloride.

[p] Tested as the monosulfate.

[q] This represents the mesityl group.

[r] Tested as the monosodium salt.

[s] Tested as the mononitrate.

[t] Dog died.

[u] 7-Chloro-4-(4-diethylamino-1-methylbutylamino)quinoline, salt with two formula weight 8-hydroxy-7-iodo-5-quinolinesulfonic acid.

[v] 4-(7-Chloro-4-quinolylamino)-α-diethylamino-*o*-cresol, salt with two formula weight 8-hydroxy-7-iodo-5-quinolinesulfonic acid.

[w] 5-Chloro-7-(diethylaminomethyl)-8-quinolinol, salt with two formula weight 8-hydroxy-7-iodo-5-quinolinesulfonic acid.

[x] See also compounds 5 and 6 in Table 1.

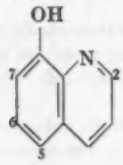
[y] Tested in a medium consisting of gastric mucin and whole egg infusion.

[z] Tested in the absence of liver extract (see also footnote "a").

extensively in treating human intestinal amebiasis and as reference drugs for experimental studies (*e.g.* Jones, 1946; Anderson and Hansen, 1950; Thompson *et al.*, 1950; and Luttermoser *et al.*, 1952). Compound 26 was reported to be clinically active by Wenger (1952). In addition, several other simple halogenated derivatives were found to be active. Thus, 5,7-dichloro-(24), 5,6,7-trichloro-(25), 5,7-dibromo-(27), 5-fluoro-7-iodo-(28), and 7-chloro-5-[(1-piperidyl)methyl]-8-quinolinol (32) were amebicidal *in vitro* at concentrations of 6–200 μg./ml., and all were active in rats; compounds 24, 25, and 27 possessed curative action in dogs. The 8-hydroxy-7-iodo-5-quinolinesulfonic acid salts (31 A, B and C) of chloroquine, amodiaquin, and 5-chloro-7-diethylaminomethyl-8-quinolinol did

TABLE 2

Halogenated 8-quinolinols.[a] Screening test results against E. histolytica *in vitro and in rats*



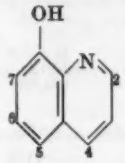
No.	Substituents	*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
			% in diet	Mg./Kg. daily, 7 days		Suppression of infection[d]	Cured[e]
24	5—Cl; 7—Cl	8[b]	0.5[z]		5	80	80
			0.25	322	10	34	15
25	5—Cl; 6—Cl; 7—Cl	167[b]	1.0[••]				
		(>2000[b])	0.5	570	4	100	100
			0.25	256	8	100	100
			0.125	154	15	72	69
			0.0625	75	11	88	73
			0.03125	42	15	49	56
26	2—CH_3; 5—Cl; 7—Cl	6[b]	0.5	364[••]	3	100	100
			0.25	131[•]	10	77	70
			0.125	124	10	38	29
27	5—Br; 7—Br	75[b]	0.5	561†	5	71	0
			0.25	314	5	85	20
			0.125	161	5	67	9
28	5—F; 7—I	110 ± 90[b,s]	1.0	1112	5	95	80
		(>2000[b])	0.475	621	10	77	77
			0.25	378	5	27	33
29	5—Cl; 7—I (Vioform)	25	0.5	401†	40	97	92
		(30[b])	0.25	279	15	50	57
30	5—I; 7—I (Diodoquin)	750[b]	1.0	1316	33	96	97
		(1500[b])	0.5	604	15	88	73
31	5—SO_3H; 7—I[r] (Chiniofon)	188[b]	1.0	1358	15	93	87
		(4000[b])	0.5	639	26	90	70
			0.25	311	19	54	53
31A	5—SO_3H; 7—I·½ $C_{18}H_{16}ClN_3$[u] (Resotren)		0.125	109†	10	18	24
31B	5—SO_3H; 7—I·½ $C_{20}H_{22}ClN_3$[v]	750[b]	0.125	128†	5	12	0
		(>2000[b,y])					
31C	5—SO_3H; 7—I·½ $C_{16}H_{17}ClN_2O$[w]	4[b]	0.125	145†	16	38	48
		(1500[b,y])					
32	5—$CH_2N(CH_3)5$; 7—Cl[t]	19	0.4	507	5	100	100
		(110 ± 90)	0.2	280	10	0	10
33	5—NH_2; 7—I	94[b]	0.5	508	4	40	6
34	5—NO_2; 7—I	375	0.125	92[••]	2	0	0

Footnotes—see Table 1.

not show promising therapeutic activity in rats. The poor performance of compound 31A in rats was not consistent with its reputed efficacy in human intestinal amebiasis. (Halawani *et al.*, 1953; Bannerjie, 1953; Chaudhuri *et al.*, 1954). The high activity *in vitro* and *in vivo* of the non-iodinated compounds 24, 25, 26 and 27 demonstrates that iodine is not an essential substituent; this fact will be given further consideration in the discussion. The halogenated 8-quinolinols as a group were rather strongly bound by mucin.

TABLE 3

Miscellaneous 2,4,5,6 and 7-substituted 8-quinolinols. Screening test results against E. histolytica in vitro *and in rats*



No.	Substituents	*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
			% in diet	Mg./Kg. daily, 7 days		Suppression of infection[d]	Cured[e]
35	2—CH_3	23[b]					
36	6—CH_3	5[b]	0.5	618	5	45	40
			0.25	359	5	27	0
37	7—CH_3	9[b]	0.5**				
			0.25	314	9	18	0
38	5—CH_3; 7—CH_3	19	1.0	776*	3	36	24
			0.5	464†	5	55	60
39	2—C_6H_5	47[b]					
40	2—[$C_6H_2(CH_3)_3$,2,4,6[q]	>2000[b,s] (>2000[b])					
41	2—(C_6H_4—*o*—OH)	1100 ± 900[b] (>2000[b])					
42	7—COOH	19	1.0	1307	4	54	25
			0.5	606	5	47	40
43	7—$COOC_2H_5$	110 ± 90[b,s] (110 ± 90[b])	0.5	513	5	5	0
44	4—(NHC_6H_4—*p*—Cl)[k]	110 ± 90[b,s] (>2000[b])	0.1	156	8	0	0
45	4—$NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$[l]	100 ± 900[b] (>2000[b])	0.5	670	4	0	0
			0.25	294	4	0	13
46	4—(NHC_6H_4—*p*—Cl); 7—$CH_2N(CH_3)_2$	1110 ± 90[b,s] (1100 ± 900[b])	0.125**				
			0.0625	58	9	27	12
47	5—NO_2; 7—NO_2	110 ± 90[b,s] (110 ± 90[b])	0.5	527**	4	72	44
			0.25	255*	3	9	0
48	7—[$CH(C_6H_5)NHC_6H_5$]	>2000[b] (>2000[b,y])					
49	7—[$CH(C_6H_5)NHC_6H_4$—*o*—OCH_3]	1500[b] (1100 ± 900[b,y])					
50	7—[$CH(C_6H_5)NHC_6H_4$—*m*—Cl]	1500[b] (>2000[b,y])					
51	7—[$CH(C_6H_5)NHC_6H_4$—*p*—NO_2]	>2000[b] (>2000[b,y])					

Footnotes—see Table 1.

TABLE 4

7-Mono and dialkylaminomethyl-8-quinolinols. Screening test results against E. histolytica *in vitro and in rats*

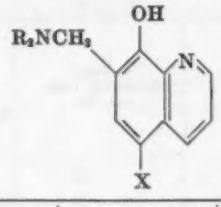
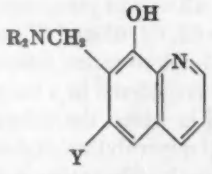
No.	Substituents		*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
	X	R_2N		% in diet	Mg./Kg. daily, 7 days		Suppression of infection[d]	Cured[e]
								
52	H	$(CH_3)_2N$—[k]	110 ± 90[b,c]	0.5	474*	4	100	100
			(1100 ± 900[b])	0.25	304	4	83	67
				0.125	170	5	0	0
53	H	$(C_2H_5)_2N$—[l]	110 ± 90	0.4	402†	5	100	100
			(1100 ± 900)	0.2	191†	5	23	20
				0.1	124	5	35	20
54	H	$(CH_3)_2N(CH_2)_2$-NH—[l]	110 ± 90	0.5	400†	5	10	0
			(1100 ± 900[b])	0.25	238	5	0	0
55	CH_3	$(C_2H_5)_2N$—[m]	1100 ± 900[a]	0.2	263	7	0	0
			(1100 ± 900)					
56	CH_3	$(CH_2)_5N$—[g,n]	38[b]	0.2	208†	17	7	25
			(1100 ± 900)					
57	CH_3	$HOCH_2CH_2$-$(C_2H_5)N$—[l]	8	0.25	230†	5	0	0
			(1100 ± 900)					
58	CH_3	$C_6H_5N[CH_2$-$CH_2]_2N$—[j]	>2000[b]					
			(>2000[b])					
59	C_2H_5	$(CH_2)_5N$—[g,m]	110 ± 90	0.25	316	4	23	50
			(1100 ± 900)					
60	$CH_2C_6H_5$	$(CH_2)_5N$—[g,n]	110 ± 90[b]					
			(1100 ± 900[b])					
61	F	$(C_2H_5)_2N$—	38	0.32	296†	4	41	50
62	Cl	$(CH_3)_2N$—[k]	38	0.4	488†	9	31	33
			(1100 ± 900)	0.2	251†	10	0	16
				0.1	122	10	0	0
				0.05	58	5	0	0
63	Cl	$(C_2H_5)_2N$—[l]	6	0.39	506*	9	100	100
			(56)	0.2	218	5	100	100
				0.1	119	19	48	57
				0.05	62	19	45	51
				0.025	27	10	17	0
64	Cl	$(CH_3)_4N$—[h,k]	12	0.4	480	10	43	37
			(1100 ± 900[b])					
65	Cl	$(CH_2)_5N$—[g,k]	19[b]	0.4	430†	9	15	47
			(1100 ± 900[b])	0.2	225†	10	12	40
				0.1	126	9	15	33
				0.05	58	10	0	0

TABLE 4—*Continued*

No.	Substituents		*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
	X	R_2N		% in diet	Mg./Kg. daily, 7 days		Suppression of infection[d]	Cured[e]
66	Cl	$HOCH_2CH_2(C_2H_5)N$—[l]	19[b]	0.2	299	5	100	100
			(1100 ± 900[b])	0.1	120	9	22	41
				0.05	64	4	0	0
67	Cl	$(HOCH_2CH_2)_2N$—[l]	75	0.2	280	5	71	80
			(1100 ± 900[b])	0.1	133	17	29	15
68	Cl	$O(CH_2CH_2)_2N$—[l]	8	0.4	537*	4	60	67
			(1100 ± 900[b])	0.2	270	15	25	29
69	Cl	$[CH_3(CH_2)_{14}CO_2(CH_2)_2]_2N$—[k]	>2000[b]	1.6	2150	5	0	0
			(>2000[b])	0.8	1020	5	0	0
70	Cl	$(CH_3)_2N(CH_2)_2NH$—[l]	75	0.2	224	5	0	20
			(110 ± 90)					
71	Cl	$(C_2H_5)_2N(CH_2)_2NH$—[l]	24	0.25	253	10	55	56
			(750[r])					
72	Br	$(C_2H_5)_2N$—[l]	19[b]	0.4	423†	10	57	47
			(110 ± 90[b])	0.2	231†	5	54	52
73	$COCH_3$	$(CH_2)_5N$—[s]	>2000[b,s]					
			(>2000[b])					
74	COC_6H_5	$(CH_2)_5N$—[s,n]	110 ± 90[b]	0.35	316†	5	0	0
			(1100 ± 900[b])					
75	NH_2	$(C_2H_5)_2N$—[o]	500	0.25	241*	10	13	20
76	NH_2	$(CH_2)_5N$—[s,o]	75[s]					
77	$NHCOCH_3$	$(C_2H_5)_2N$—[l]	1100 ± 900					
			(>2000[b])					
78	$NHCOCH_3$	$(CH_2)_5N$—[s]	1100 ± 900[s]					
			(>2000)					
79	NO_2	$(C_2H_5)_2N$—	75	0.5	**			
				0.25	**			
				0.125	120*	5	0	0



	Y	R_2N						
80	Cl	$(CH_2)_5N$—[s]	110 ± 90[l,s]	0.5	540*	5	100	100
			(110 ± 90)	0.25	263	5	40	50
				0.125	154	5	33	25
81	Cl	$(C_2H_5)_2N$—	19[l]	0.4	188**			
			(1100 ± 900)	0.2	239*	6	69	60
				0.1	114	5	0	11

Footnotes—see Table 1.

Miscellaneous 2,4,5,6 and 7-substituted 8-quinolinols. Seventeen compounds were tested (see Tables 3 and 7). Methyl groups on the quinoline nucleus appeared to exert little effect on activity *in vitro*, since the methyl substituted compounds 2, 35, 36, 37 and 38 had the same high order of activity as 8-quinolinol (1) itself; 5,7-dimethyl-8-quinolinol (38) was active in rats. High activity *in vitro* was also observed with 7-carbethoxy-(43) and 7-carboxy-8-quinolinol (42); the latter was active in rats, but ineffective in dogs. Although 2-phenyl-8-quinolinol (39) possessed good effect *in vitro*, activity was lost when the phenyl group was replaced by a mesityl (40) group. The 4-anilinoquinolinols 44 and 46 possessed good activity *in vitro* but neither was effective in rats; 5,7-dinitro-8-quinolinol (47) was active *in vitro* and effective, although toxic, in rats.

7-Mono and dialkylaminomethyl-8-quinolinols. Thirty compounds of this type (Burckhalter and Edgerton, 1951; Edgerton and Burckhalter, 1952) were tested *in vitro* and in rats and dogs (see Tables 4 and 7). The general structure of the 5-substituted-7-mono and dialkylaminomethyl-8-quinolinols is indicated at the top of Table 4.

Compounds of this formula, when X is H and R_2N is dimethylamino (52) or diethylamino (53), were active both *in vitro* and in rats; compound 53 was effective in dogs. When the hydrogen atom was replaced by an alkyl group (compounds 55 through 60), the activity *in vitro* varied from 8 to >2000 μg./ml., depending upon the side-chain. Compound 59 possessed slight activity in rats, while 55, 56 and 57 failed to exert appreciable therapeutic effect.

The introduction of a fluorine (61) or bromine (72) atom at X, where R_2N represents diethylamino, yielded compounds with high activity *in vitro*; both were active in rats, and the bromo derivative cured amebic colitis in dogs.

Peak activity in this series was observed when a chlorine atom was inserted at X. Among ten 5-chloro-8-quinolinols carrying various side-chains, 6 were effective *in vitro* in concentrations of <25 μg./ml. These were diethylamino-(63), pyrrolidyl-(64), piperidyl-(65), ethyl β-hydroxyethylamino-(66), morpholinyl-(68), and β-diethylaminoethylamino-methyl (71) derivatives. When these 6 compounds were tested in rats, all except compounds 64 and 65 were active at well tolerated doses; compounds 62, 63, 65 and 67 cured dogs (see Table 7). The most promising member of the 5-chloro series, namely 5-chloro-7-diethylaminomethyl-8-quinolinol (63), will be considered in a later section of this paper.

Two compounds were studied in which the chlorine atom was moved to the 6-position, namely, 6-chloro-7-(1-piperidyl)methyl-(80) and 6-chloro-7-diethylaminomethyl-8-quinolinol (81); both were active *in vitro* and in rats.

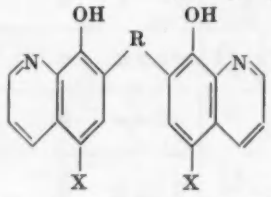
Introduction of the benzoyl (74), amino (75, 76), acetylamino (77, 78), and nitro (79) groups in the 5-position and of various Mannich side-chains in the 7-position yielded compounds that were active *in vitro*, but 74, 75 and 79 were ineffective in rats.

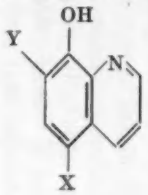
Twenty-one of these active Mannich derivatives of 8-quinolinol were tested in mucin; activity was reduced in 19 cases, indicating that this series is rather uniformly bound by mucin.

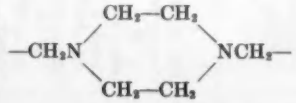
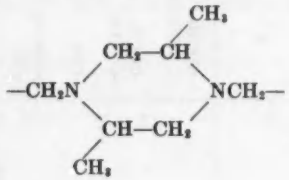
Quinolyl-8-quinolinols. Data on these are listed in Table 5. It is noteworthy

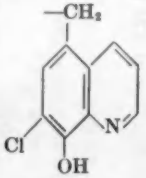
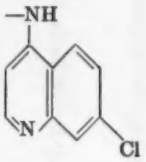
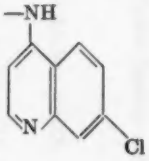
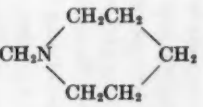
TABLE 5

Quinolyl-8-quinolinols. Screening test results against E. histolytica in vitro





No.	X	R	*In Vitro*[a] Amebicidal Conc. μG./Ml.
82	Cl	$-CH_2-$	1100 ± 900[b,a] (>2000[b])
83	Cl		>2000[b,a] (>2000[b])
84	Cl		>2000[b,a] (>2000[b])
85	NH_2	$-CH_2-$	75[b] (1100 ± 900[b])

No.	X	Y	*In Vitro*[a] Amebicidal Conc. μG./Ml.
86		Cl	>2000[b] (>2000[b])
87		H	150[b] (1100 ± 900[b])
88			110 ± 90[a] (>2000[b])

Footnotes—see Table 1.

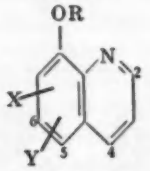
that while 5-chloro-8-quinolinol (5 in Table 1) was highly active *in vitro* and in rats, three compounds (82, 83 and 84) in which two 5-chloro-8-quinolinol moieties were joined through the 7-positions possessed little or no activity *in vitro*. In contrast, the joining of two active 5-amino-8-quinolinol moieties (16 in Table 1) through a methylene bridge yielded a compound (85) which appeared to be more active. These opposing observations discourage predictions regarding activity for compounds formed by joining two active moieties.

The combination of the 8-quinolinol nucleus and the 4-aminoquinoline nucleus as in compounds 87 and 88 yielded products with good activity *in vitro*.

Ethers and esters of 8-quinolinols. Ten ethers and esters of 8-quinolinols were tested (Table 6). *In vitro*, the acetate esters 89, 91 and 92 were as active as the corresponding 8-quinolinols (1, 7, 17 in Table 1), an observation not unexpected since acetates are easily hydrolyzed. The ethers 90, 93, 94, 95, 97 and 98 were also active *in vitro*. It remains to be determined whether the ethers and esters are active *per se* or are cleaved *in vitro* to yield the parent 8-quinolinols. In general, the above results are not in complete agreement with those of Wieder (Schönhöfer, 1948) on related compounds (see Table 9), who concluded that activity was almost completely lost by etherization and was reduced by acylation.

TABLE 6

Ethers and esters of 8-quinolinols. Screening test results against E. histolytica *in vitro and in rats*



No.	Substituents		*In Vitro*[a] Amebicidal Conc. μG./Ml.	Drug		No. of Rats	Therapeutic Effects (%)	
	X, Y	R		% in diet	Mg./Kg. daily 7 days		Suppression of infection[d]	Cured[e]
89	5—H	—$COCH_3$	8	0.5	465	4	32	0
90	5—H	—CH_2COOH[k]	1100 ± 900[a]	0.5	255[e]	2	17	0
			(1100 ± 900)	0.34	464	3	10	0
91	5—$COCH_3$	—$COCH_3$	2[b]	0.05	699	5	71	80
92	5—$NHCOCH_3$	—$COCH_3$	1100 ± 900[b,a] (>2000[b])					
93	5—NO_2	—CH_3	110 ± 90[b, a] (1100 ± 900[b])	0.5	376[e]	15	46	28
94	5—NO	—$CH(CH_3)_2$[k]	2[f,b,a]					
95	5—I; 4-NHC_6H_5—p-OH-m-$CH_2N(C_2H_5)_2$	—CH_3[l]	110 ± 90[b] (>2000[b])					
96	5—Cl; 4—NHC_6H_5—p-OH-m-$CH_2N(C_2H_5)_2$	—CH_3[l]	>2000[a] (1100 ± 900)	0.125†	193	5	0	0
97	6—$NH(CH_2)_3N(CH_2CH_3)_2O$	—CH_3[l]	110 ± 90[a]	0.25	305†	3	12	0
			(110 ± 90)	0.125	155	5	0	20
98	2—COOH; 4—OH; 5—I	—CH_3	1100 ± 900[b,a] (>2000[b])					

Footnotes—see Table 1.

2. The effects of certain substituted 8-quinolinols in hepatic amebiasis

Data pertaining to the effects of 4 compounds are presented in Table 8. 5,7-Diiodo-8-quinolinol (30) was tested as a representative of the halogenated 8-quinolinols that are commonly used in clinical intestinal amebiasis. 7-Methyl-(37), 5-chloro-7-diethylaminomethyl-(63) and 5-bromo-7-diethylaminomethyl-8-quinolinol (72) were selected because of their high activity *in vitro*.

Compounds 30 and 37 did not appear to be effective orally in relatively large doses. Compound 72 appeared to have slight suppressive action when administered subcutaneously; these injections caused marked local irritation regularly and death in 3 of 11 animals. The effects with compound 63 will be considered

TABLE 7

8-Quinolinols. Screening test results against E. histolytica in vitro *and in dogs*

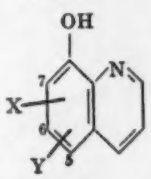
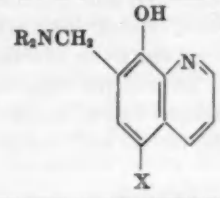
No.	X, Y	*In Vitro*[a] Amebicidal Conc. μG./Ml.	In Dogs			
			Mg./Kg. daily 10 days	No. benefit	Inf. supp.	Inf. cured
						
1	5—H; 7—H	15[b]	800		1[c]	
			200	1	1	
			50	1		
24	5—Cl; 7-Cl	8[b]	200		1[c]	1
			150		1, 1[c]	
			100		1	
25	5—Cl; 6—Cl; 7—Cl	167[b]	50			1
		(>2000[b])	25	2		1
			12.5	1		
27	5—Br; 7—Br	75[b]	200			1
			100			1
			50	1		
29	5—Cl; 7—I	25	50			2
	(Vioform)	(30[b])	25	2		
			12.5		1	
30	5—I; 7—I	750[b]	250	1		2
	(Diodoquin)	(1500[b])	200		1	
			150		2	
			63	1		
			16	1		
7	5—$COCH_3$; 7—H	4	800			1
			200		1	1
			50	1		1
42	5—H; 7—COOH	19	800†	1		
			200	1		

TABLE 7—*Continued*

No.	X	R_2N	*In Vitro*[a] Amebicidal Conc. μG./Ml.	In Dogs			
				Mg./Kg. daily 10 days	No benefit	Inf. supp.	Inf. cured
							
53	H	$(C_2H_5)_2N$—[l]	110 ± 90	100			1
			(1100 ± 900)	50	1		
				12.5	1		
62	Cl	$(CH_3)_2N$—[k]	38	25	1		
			(1100 ± 900)	12.5	1	1	1
				6.25	1		
63	Cl	$(C_2H_5)_2N$—[l]	6	50			1
			(56)	25	1		2
				12.5	2		2
				6.25	2		
64	Cl	$(CH_2)_4N$—[b,k]	12	25	1	1	
			(1100 ± 900[b])				
65	Cl	$(CH_2)_6N$—[g,k]	19[b]	12.5			2
			(1100 ± 900[b])	6.25	1		
66	Cl	$HOCH_2CH_2(C_2H_5)N$—[l]	19[b]	25	2		
			(1100 ± 900[b])				
67	Cl	$(HOCH_2CH_2)_2N$—[l]	75	50			1
			(1100 ± 900[b])	25		1	2
				12.5	1		
72	Br	$(C_2H_5)_2N$—[l]	19[b]	50			1
			(110 ± 90[b])	25		1	1

† 800 mg./kg./day orally for the first 5 days and then 600 mg./kg./day orally plus 200 mg./kg./day intramuscularly for the second 5 days.

For other footnotes—see Table 1.

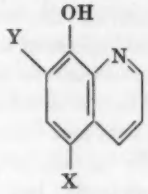
later but it may be noted here that they were not outstanding. Due to untimely deaths among each of the treated groups, which may have introduced an element of bias, the above results are only exploratory. However, the data are adequate to indicate that none of these compounds is worthy of serious interest for the treatment of hepatic amebiasis.

5-CHLORO-7-DIETHYLAMINOMETHYL-8-QUINOLINOL (COMPOUND 63) AS A POTENTIAL ANTIAMEBIC DRUG

Compound 63 (Tables 4, 7 and 8) appeared in screening tests (supra) to be one of the most promising new substances, comparing favorably with the iodinated 8-quinolinols in clinical use, namely compounds 29, 30 and 31. Consequently,

TABLE 8

Effects of various substituted 8-quinolinols against amebic hepatitis in hamsters



Comp. No.	Substituents		Route Drug Admin.	Mg./Kg. Daily, 4 Days	Treated			Untreated			% Supp. Lesion Wt.
	X	Y			Surv./total	% surv. pos. for amebae	Mean lesion wt. (G)	Surv./total	% surv. pos. for amebae	Mean lesion wt. (G)	
30	I	I	Oral	500	7/10	100	1.16	8/10	100	1.19	2.5
37	H	CH_3	Oral	500	7/10	100	1.19	8/10	100	1.19	0
63	Cl	$CH_2N(C_2H_5)_2$	Oral	200	5/10*	100	0.63	10/10*	100	1.32	52 (?)§
			Oral	100	7/10	100	1.25	9/10	100	1.21	0
			Subcut.†	50	8/9*	100	0.76	10/10*	100	1.39	45
72	Br	$CH_2N(C_2H_5)_2$	Subcut.	25-50‡	8/11	100	0.76	9/9	100	1.32	42 (?)§

* Liver tissue from these animals analyzed for drug, see text.

† Given in sesame oil.

‡ Given at the rate of 50 mg/kg/day for 1½ days and then at 25 mg/kg/day for 2½ days. Highly irritating at site of injection.

§ Deaths among the treated animals, due to drug toxicity, may have introduced bias in these results.

63 was selected for expanded studies to explore its potential usefulness in the chemotherapy of amebiasis.

In vitro activity. When tested against ameba-bacteria cultures, compound 63 was amebicidal in respective concentrations of 6 and 56 μg./ml. in the protein-deficient and the mucin media. In order to obtain more precise information as to whether it acted directly or indirectly, a coliform bacillus and an organism of the *Streptococcus faecalis* type were isolated from the cultures and tested turbidimetrically in egg yolk infusion for sensitivity to # 63. The coliform bacillus grew well in concentrations up to 20 μg./ml. but the *Strept. faecalis* type organism was inhibited at a concentration of 2.5 μg./ml., which was less than the amebicidal level. While there has been some suggestion from certain other studies that amebae depend mainly upon the coliform bacillus, these data are inadequate for proving that # 63 affected the amebae directly.

Compound 63 was amebicidal at 90 μg./ml. in tests with bacteria-free *E. histolytica-T. cruzi* cultures; these tests employed a medium preconditioned with *T. cruzi*, which would reduce the likelihood of indirect effect. However, the motility of *T. cruzi* at the amebicidal concentration of drug was poorer than in the control cultures. Thus, again, the possibility of an indirect action was not ruled out. The differences in level of activity among the 3 types of tests apparently were associated with variations in test media, culture associates, and strains of amebae.

Therapeutic effect against intestinal amebiasis in rats. The effects of # 63 when administered in the diet are shown in Table 4. These data indicate that doses of

62 to 119 mg./kg./day gave about 50 per cent effect, either in terms of the percentage of cures attained or in terms of the suppression of the average degree of infection. Intakes of 218 or 506 mg./kg./day were completely effective; evidence of intolerance occurred only at the highest of these dose levels.

Therapeutic effect against cecal amebiasis in dogs. Ten dogs with severe amebic dysentery were treated with #63 in oral doses ranging from 6.25 to 50 mg./kg./day for 10 days (Table 7). A dog that had been infected for 186 days was cured with 50 mg./kg./day. Doses of 25 mg./kg./day cured 2 dogs that had been infected for 54 and 158 days, respectively, but failed to cure one that had been infected for 67 days. Two of 4 dogs treated with 12.5 mg./kg./day were cured; the cured animals had been infected 16 and 370 days, respectively, the uncured dogs for 16 and 42 days. Doses of 6.25 mg./kg./day failed to cure 2 dogs that had been infected for respective periods of 21 and 42 days. Among the 5 dogs that were cured, the drug appeared to have rapid therapeutic effect, causing the disappearance of amebae within 2 to 4 days. No relationship was apparent between the age of infection and susceptibility to treatment.

Therapeutic effect against amebic hepatitis in hamsters. The effects of #63 when given orally or subcutaneously are summarized in Table 8. Oral doses of 200 mg./kg./day appeared to confer partial suppression of the lesions but also were toxic. Moreover, the several deaths among the treated animals may have introduced bias since it is likely that drug tolerance would be inversely related to lesion size. Oral doses of 100 mg./kg./day failed to suppress the infections and were toxic.

Administration of the drug subcutaneously with sesame oil in doses of 50 mg./kg./day conferred 45 per cent suppression, and one of the 9 animals died; this single death may have introduced a small, but hardly important, element of bias.

Observations on the physiologic disposition of #63 in animals[2]. Although no systematic study has been made concerning the fate of the compound in animals, certain observations were made in connection with chemotherapeutic studies. A modification of the methyl orange method of Brodie, Udenfriend and Dill (1947) was used in assaying biological material for #63. Specimens were examined in parallel from treated and untreated animals.

Compound 63 was given to hamsters (Table 8) orally in doses of 200 mg./kg./day or subcutaneously in doses of 50 mg./kg./day for 4 days. The daily dose was divided between early morning and late afternoon. Approximately 18 hours after the last dose, the livers were assayed for #63. No significant concentration of drug was detected following either route of administration.

A dog was given #63 orally in a dose of 25 mg./kg.; samples of blood were taken 1, 2 and 4 hours later; urine was collected by catheter 2 hours after dosing; colonic aspirates were taken 1, 2, 4 and 8 hours after dosing. No drug was detected in the blood, urine, or the first 3 colonic aspirates; however, the 8-hour colonic aspirate contained 252 μg./ml. of #63 equivalent.

[2] We are indebted to Dr. A. J. Glazko and Mr. Wesley Dill of these laboratories for chemical determinations of #63 in biological specimens.

These data, together with its greater subcutaneous effect (*supra*), indicate that # 63 is absorbed poorly from the gastro-intestinal tract. Failure to detect the drug in the livers of hamsters dosed subcutaneously indicates that it is rapidly excreted or degraded; these mechanisms would account for absence of the drug in the blood and urine of the dog in the event of appreciable absorption from the gastrointestinal tract. The apparent limited oral absorption of # 63 and its not more than transient persistence in the blood or liver help to explain its better performance against intestinal amebiasis than against hepatic amebiasis.

In view of these observations on the absorption and metabolism of compound 63, it is pertinent to note the absence of high serum levels of 5-acetyl-8-quinolinol (7 in Table 7) in dogs that had been treated orally twice daily with doses of 25, 100 and 400 mg./kg. The treatment regimen and dogs were the same as those used for chemotherapeutic tests, and serum was taken 6 hours after the 17th dose (8.5 days of treatment). Assays[3] with the $H_{37}RV$ strain of *Mycobacterium tuberculosis* showed each sample contained less than 4 μg./ml.

Toxicity of # 63 in normal animals[4]. The acute oral toxicity was determined in albino mice and rats. A single dose of drug was given by gavage; deaths were recorded during one week after dosing. The drug was given to 160 mice in dosages ranging from 94 to 748 mg./kg. and to 210 rats in dosages ranging from 59 to 375 mg./kg. The MTD and LD_{50} in mice were, respectively, 112 and 244 mg./kg. of body weight. The MTD and LD_{50} in rats were, respectively, 78 and 169 mg./kg.

The chronic oral tolerance in mice was determined by feeding various concentrations of the drug in the diet for 4 weeks. The MTD for this period (50 mice) was estimated to be 684 mg./kg./day.

The toxicity of # 63 also was studied in 4 dogs. The drug was given orally in in gelatin capsules twice daily 5 days a week. One dog each was given daily doses of 7.8, 15.7, 31.4 and 62.7 mg./kg. The dogs that received the first 3 dose levels were treated for 6 weeks; they showed no toxic reactions, no significant variations in blood or urine, and only a slight weight loss. The dog given 62.7 mg./kg./day vomited frequently, developed anorexia and diarrhea, and died after 11 days of dosing.

Significant histopathology occurred only in dogs and consisted chiefly of mild to moderate kidney and liver damage, roughly paralleling the drug dose. Evidence of gastric irritation occurred only in the dog receiving the highest dose level.

DISCUSSION

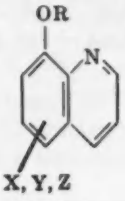
With the exception of the three iodinated compounds used in the treatment of human amebiasis, little has been published on the antiamebic action of 8-quinolinols. This is rather surprising, since these substances were introduced as

[3] We are indebted to Dr. A. C. Bratton, Jr. for these assays.

[4] The authors are indebted to Dr. J. K. Weston and his staff of these laboratories for permission to include these data.

TABLE 9

Literature survey on the antiamebic action of 8-quinolinols exclusive of those in clinical use



Substituents		*In Vitro* Amebicidal Conc., μG./Ml.	Reference
R	X, Y, Z		
H	H[a]	100	Anderson et al. (1931)
H	5—Cl	100	Idem
		10–20	Schönhöfer (1948)
		b, c	Anderson and Hansen (1950)
H	5—Cl; 7—$CH_2CH{=}CH_2$	d	Schönhöfer (1948)
H	5—Cl; 7—$CH_3CH{=}CHCH_2$	e	Idem
H	5—Cl; 6—Cl; 7—Cl	b	Anderson and Hansen (1950)
H	5—Br; 7—Br	b	Idem
H	5—Br; 7—I	b	Idem
H	5—Cl; 7—SO_3H	e	Schönhöfer (1948)
H	5—$CO(CH_2)_6CH_3$	100	Anderson and Alles (1945)
H	5—Cl; 7—NO_2	e	Schönhöfer (1948)
—C_2H_5	5—Cl	f	Idem
—$CH_2CH{=}CH_2$	5—Cl	f	Idem
—$CH_2C_6H_5$	5—Cl	f	Idem
—$CH_2COC_6H_5$	5—Cl	f	Idem
—$CH_2C(=NOH)C_6H_5$	5—Cl	f	Idem
—CH_2COOH	5—Cl	f	Idem
—$CH_2COOC_2H_5$	5—Cl	f	Idem
—CH_2CONH_2	5—Cl	f	Idem
—$CH_2CON(CH_3)_2$	5—Cl	f	Idem
—CH_2CSNH_2	5—Cl	f	Idem
—$CH_2CONHNH_2$	5—Cl	f	Idem
—CH_2CN	5—Cl	f	Idem
—$CH_2CH_2N(C_2H_5)_2$	5—Cl	20	Leake (1932)
		20–100	Bradin and Hansen (1950)
		10	Hansen and Bennett (1952)
		100	Balamuth (1953)
—$COCH_3$	5—Cl	g	Schönhöfer (1948)
—$COOC_2H_5$	5—Cl	g	Idem
—COC_6H_5	5—Cl	g	Idem
—COC_6H_4-*o*-OH	5—Cl	g	Idem
—$CON(CH_3)_2$	5—Cl	g	Idem
—$COCH_3$	6—NO_2	e	Swiss Patent 294,172 (1954)

TABLE 9—*Continued*

[a] Tested as the sulfate salt (chinosol).

[b] Less than half as active as emetine, which was effective at 2.5 to 10 μg./ml.

[c] Ineffective against *E. histolytica* in monkeys at doses of 300 mg./kg. daily for 5 days (Anderson and Hansen, 1950).

[d] Possessed nearly the same activity as 5-chloro-8-quinolinol but was 10 times less toxic in mice.

[e] Precise activity not indicated.

[f] Amebicidal action was completely or almost completely lost by etherization. Precise activity not indicated.

[g] The acyl derivatives showed a weaker activity and lower toxicity than 5-chloro-8-quinolinol. Precise activity not indicated.

amebicides many years ago—sodium 7-iodo-8-quinolinol-5-sulfonate by Mühlens and Menk (1921), 5-chloro-7-iodo-8-quinolinol by Anderson and Koch (1931), and 5,7-diiodo-8-quinolinol by Tenney (1936). Wenger (1952) reported good results with 5,7-dichloro-2-methyl-8-quinolinol in chronic intestinal amebiasis, but restricted parasitological follow-up in a small series of patients makes it difficult to assess the value of the drug. A summary of published laboratory observations concerning the antiamebic activity of other 8-quinolinols is given in Table 9. In addition to the compounds tabulated, a number of other 8-quinolinols have been prepared as amebicides (Ghosh *et al.*, 1945; Ghosh, 1947; Hoffmann, 1947, and Coll and Coll, 1951), but no pharmacological results were reported.

In the present study, activity in the 8-quinolinol series was found to be widespread. Thus, 84 of the 98 compounds were amebicidal *in vitro* at concentrations of 2000 μg./ml. or less; 16 at 200–2000 μg./ml.; 45 at 20–200 μg./ml.; and 23 at <20 μg./ml.

Of the 84 compounds that were active *in vitro*, 60 were tested against intestinal infections in rats. Twenty-nine of these reduced the ADI in rats 50 per cent or more and/or cleared 50 per cent or more of the animals. The data in Table 10 strongly indicate that in the 8-quinolinol series, a definite correlation exists between high activity *in vitro* and activity in rats.

Fifty-five of these active compounds were examined in the presence of gastric mucin. The same order of activity was maintained by 24 per cent, while 76 per cent appeared to be significantly less active, indicating that the 8-quinolinols as a group are rather strongly bound by mucin. Thirty-eight of the compounds tried

TABLE 10

Correlations between activities of sixty 8-quinolinols and derivatives in vitro *and in rats*

In Vitro, Amebicidal Concentration (μG./Ml.)	In Rats	
	No. active / Total	% active
>2000	0/3	0
200–2000	1/6	17
20–200	15/32	47
<20	13/19	69

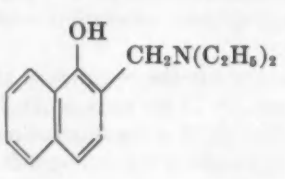
in rats were tested in mucin. Among 19 that were inactive in rats, activity of 12 was reduced by mucin, indicating that mucin binding may be one reason for a lack of *in vivo* activity. However, poorer effect in the presence of mucin also occurred with 15 of 19 compounds that had definite therapeutic effect. Hence, reduced activity by mucin was not an adequate criterion for rejecting compounds for trial *in vivo*.

Sixteen compounds were tested in dogs and 14 were found to have therapeutic effect. Correlations of activity *in vitro* and in rats with activity in dogs were good. Twelve were tested in rats, and 10 possessed therapeutic effect. All of the 14 compounds active in dogs were effective *in vitro* at concentrations of <200 μg./ml., with the exception of 5,7-diodo-8-quinolinol; 6 were amebicidal at <20 μg./ml.

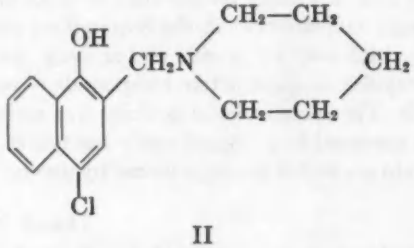
Although we assume that relationships between the size and nature of substituents on the 8-quinoline nucleus and activity must exist, no general relationship is apparent at the present state of our knowledge. However, the introduction of extremely large groups such as nonyl(13), hexadecyl(4), and hexadecanoyl (10) in position 5 destroys activity.

Such factors as chelation, ionization, and oil/water partition coefficients (Albert *et al.*, 1954) probably exert a strong influence on structure-activity relationships in this series, but the absence of parallel physical studies makes it impossible to evaluate these effects. Albert *et al.* (1947) observed that 5-chloro-7-iodo-8-quinolinol possesses antibacterial action as well as good chelating properties, but that 7-iodo-8-hydroxy-5-quinolinesulfonic acid has neither. This led them to conclude that while chelation is necessary for antibacterial action among the 8-quinolinol drugs, it is not the source of their efficacy as amebicides.

When the 8-quinolinol nucleus in compounds 53 and 65 was replaced by the 1-napthol nucleus (compounds I and II), activity dropped sharply. *In vitro*, I was amebicidal at a concentration of 1100 ± 900 μg./ml., but was inactive in rats at the MTD; II was inactive *in vitro*.



I



II

It may be helpful to consider the mechanisms of antiamebic action among the substituted 8-quinolinols. Although several mechanisms are probably represented among the compounds included in this report, one of the first considerations is whether they kill amebae by inhibiting the supporting micro-organisms or whether they kill directly. The possibility of indirect action is suggested since many 8-quinolinol derivatives are effective against bacteria; several have been used clinically as topical germicides (von Oettingen, 1933). Recently, Albert and

co-workers (e.g. Albert *et al.*, 1947) observed moderate to high antibacterial action *in vitro* by 8-quinolinol and the following derivatives: 2-methyl-(35), 5-methyl-(2), 5-*n*-propyl-, 5-chloro-(5), 7-chloro-, 5,7-dichloro-(24), 5-nitro-(21), 5-chloro-7-iodo-(29), 7-amino-, and 5,7-diamino-. 5,7-Diiodo-8-quinolinol (30) was not tested due to its low solubility in water; chiniofon (31) killed *Clostridium welchii* at 312 μg./ml. but was ineffective in high concentrations against 5 other species of bacteria. Further evidence of antibacterial action by several of the present compounds is available from unpublished studies in these laboratories; the effects of compound 63 on certain bacteria already have been described. Nevertheless, considerable evidence points to direct antiamebic activity by these compounds. In addition to the results of our *in vitro* tests, ostensible direct action has been reported for 5,7-diiodo-8-quinolinol by Nakamura and Anderson (1951) and by Bradner and Rawson (1951); for 5-chloro-7-iodo-8-quinolinol by Bradner and Rawson (1951); and for 5-chloro-7-iodo-8-diethylaminoethoxyquinoline by Balamuth (1953), Bradin and Hansen (1950), Hansen and Bennett (1952), and Nakamura and Anderson (1951). In summary, the possibility of indirect action by various members of the series cannot be denied, but there appears to be adequate basis for assuming that many substituted 8-quinolinols are directly amebicidal.

The antiamebic activity of the iodinated 8-quinolinols (29, 30 and 31) has been assumed to result from the evolution of iodine (e.g. Albert *et al.*, 1947, and Fieser and Fieser, 1950). Apparently this assumption developed from such considerations as: (1) Giemsa's (1928) statement that the bromine and chlorine analogs of sodium 7-iodo-8-quinolinol-5-sulfonate (chiniofon) were not effective clinically; (2) the concept on the part of some investigators that these compounds in water readily liberate iodine (e.g. Albert *et al.*, 1947) even though inconsistent results have been reported by the several workers who have studied this problem (von Oettingen, 1933); (3) the finding by Knight *et al.* (1949) that the *in vitro* action of serum from patients treated orally with the above drugs was proportional to the amount of serum iodine. Unfortunately in the last study no consideration was given to the 8-quinolinol moiety, which proved in the present study to be highly active *in vitro*.

The foregoing evidence is not conclusive that iodine is responsible for the activity of these drugs. Nevertheless, it is hardly coincidental that the 8-quinolinols of established clinical value in amebiasis all contain iodine. Alternately, we propose that the iodine substituent reduces both absorption from the gastrointestinal tract and general host toxicity, but contributes little to the enhancement of amebicidal action. Thus, the chlorine and bromine substitution products of sodium 7-iodo-8-quinolinol-5-sulfonate (31) were more irritating to the intestine than was the 7-iodo compound (Giemsa, 1928); 5,7-diiodo-8-quinolinol (30) was much less toxic in rats and dogs in this study than the 5,7-dichloro-(24) or the 5,7-dibromo-(27) analogs, and it was less active *in vitro*. Further, many additional non-iodinated 8-quinolinols proved to be effective in the present work both *in vitro* and *in vivo*; references to related observations in other laboratories are given in Table 9. Finally, we failed to cure amebic dysentery in dogs by giving large oral doses either of elemental iodine (200 mg./kg./day for 10 days) or of

sodium iodide (193 mg./kg./day for 5 days followed by 385 mg./kg./day for 5 days).

The activity of compound 63 (5-chloro-7-diethylaminomethyl-8-quinolinol) *in vitro* and in experimental intestinal amebiasis is equivalent to or exceeds that of the clinically-used iodinated-8-quinolinols (compounds 29, 30, 31). Furthermore, toxicity studies in several species of animals indicated that the drug may be safely tried in man. Therefore clinical trial has been initiated under the auspices of the Department of Clinical Investigation of Parke, Davis and Company. The details concerning these trials are not available at this time; however, the drug has exhibited therapeutic effect in human intestinal amebiasis, although it remains to be determined whether it has merit over the other available drugs.

SUMMARY

In a search for more effective amebicides, 98 substituted 8-quinolinols were tested against *Endamoeba histolytica*; all were examined *in vitro*, 63 against intestinal amebiasis in rats, 14 against intestinal amebiasis in dogs, and 4 against hepatic amebiasis in hamsters.

Many of the compounds were effective *in vitro* and against intestinal infections, but none of those tested against hepatic infections was encouragingly effective in nontoxic doses. Consideration of the manifest structure-activity relationships showed that activity was associated with a wide variety of substituents, particularly in the 5-, 6- or 7-position of the 8-quinolinol nucleus. Many of the active compounds did not contain iodine, thus casting doubt on the existing theory that iodine *per se* is responsible for the activity of the various iodinated-8-quinolinols in clinical use as amebicides.

Although most of the compounds were less active *in vitro* in the presence of gastric mucin than in its absence, many of them still were active *in vivo*. Therefore, susceptibility to mucin binding alone does not appear to be an adequate basis for rejecting compounds at the *in vitro* level. Good correlation was observed between high activity *in vitro* and therapeutic effect *in vivo*.

5-Chloro-7-diethylaminomethyl-8-quinolinol proved to be one of the most promising compounds. Its chemotherapeutic effects and toxicity in several species of animals are described, and a few observations are presented on its physiological disposition in animals.

ACKNOWLEDGMENT

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HUMAN TRYPANOSOMIASIS IN NORTHEASTERN LIBERIA

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Cases of human trypanosomiasis were first reported from the Ganta Dispensary by Harley (1933). From that time to the present, trypanosome infections have continued to represent an important segment of the patients seeking treatment at the Dispensary. Over the 10-year period 1944 to 1953, a total of 5,214 or roughly 10 per cent of all patients seen in the outpatient department were treated for trypanosomiasis. This report analyses the data represented by these cases.

MATERIALS AND METHODS

Only cases from the Sanoquelle District of Liberia are included here. This District comprises the tip of the Central Province which projects into French Territory, bounded on the west by French Guinea and on the east by the Ivory Coast. Trypanosome cases coming from French Territory, of which a number are treated each year, have not been included, nor have cases from other parts of Liberia.

Diagnosis is based on clinical and laboratory examinations. The laboratory staff is well-trained and competent in the technique of lymph node puncture, and the examination of node juice and thick blood films for trypanosomes. Unfortunately it has not been possible to carry out spinal fluid taps on more than a few of our patients, and this has undoubtedly lowered the rate of proven parasite-positive cases.

The clinical picture, while varied, is nevertheless fairly typical, and a clinical diagnosis alone may be depended upon to be accurate in the large majority of cases. Clinical signs most commonly seen in our patients are: enlarged cervical lymph nodes; mask-like facies; impaired neuro-muscular coordination, usually affecting locomotion; tremors of hand and tongue, or generalized; tension tremors; choreiform jerking and facial twitching; disturbed emotional states including euphoria, depression and mania; and finally, stupor. The most common symptom is sleepiness by day, often associated with restlessness and insomnia at night. Headache and generalized itching of the skin are also frequent complaints. In view of the difficulty of obtaining a reliable history from our patients, and the complex of diseases from which most of them suffer, not too much importance is given to symptoms aside from the complaint of sleepiness by day.

Symptoms of the earlier stages of infection are not usually severe enough to drive the patient to seek aid at the clinic. Therefore a majority of patients reaching the Dispensary show signs of advanced disease with involvement of the central nervous system. Whenever the spinal fluid has been examined in these cases, almost invariably we have found an increased cell count and albumin content; frequently trypanosomes are found as well.

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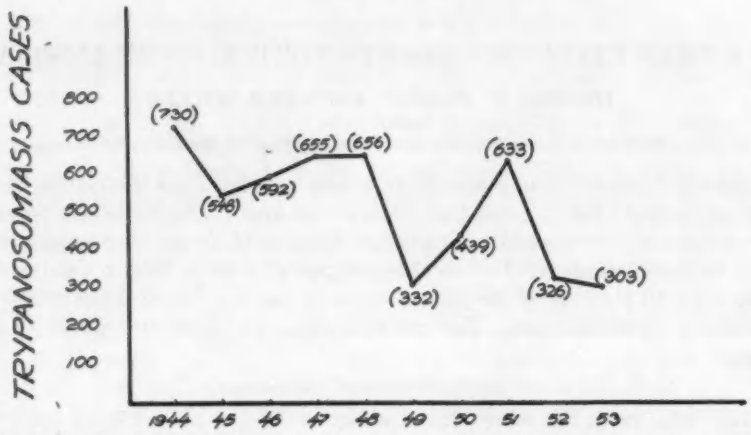

FIG. 1. Human trypanosomiasis cases treated annually at the Ganta Dispensary, Liberia, for the ten-year period 1944–1953.

RESULTS

Annual rates. Fig. 1 shows the number of cases of trypanosomiasis treated at the clinic each year from 1944 through 1953. The total number of cases seen in the five-year period 1944 through 1948 is greater than those for 1949 through 1953. Nevertheless, the cases seen in 1951 compare with any figure for the previous years.

Age distribution. As most of the patients do not know their age, this is esti-

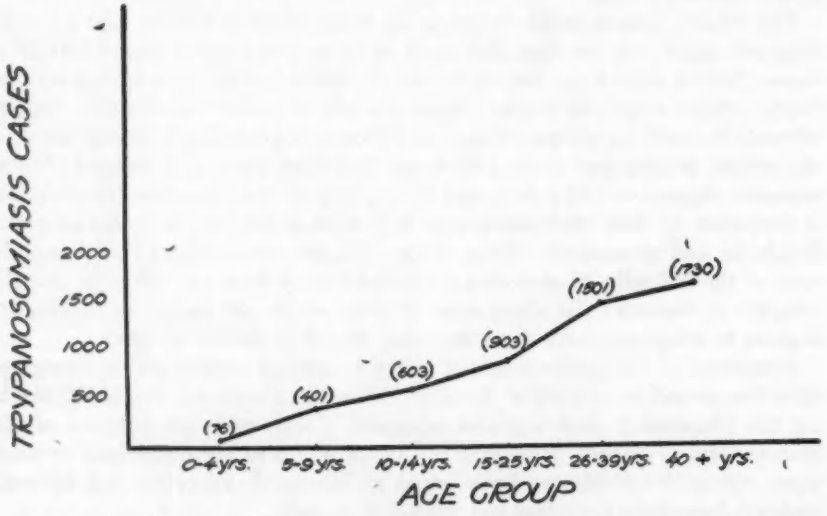

FIG. 2. Age distribution of trypanosomiasis cases attending the Ganta Dispensary, Liberia, 1944–1953.

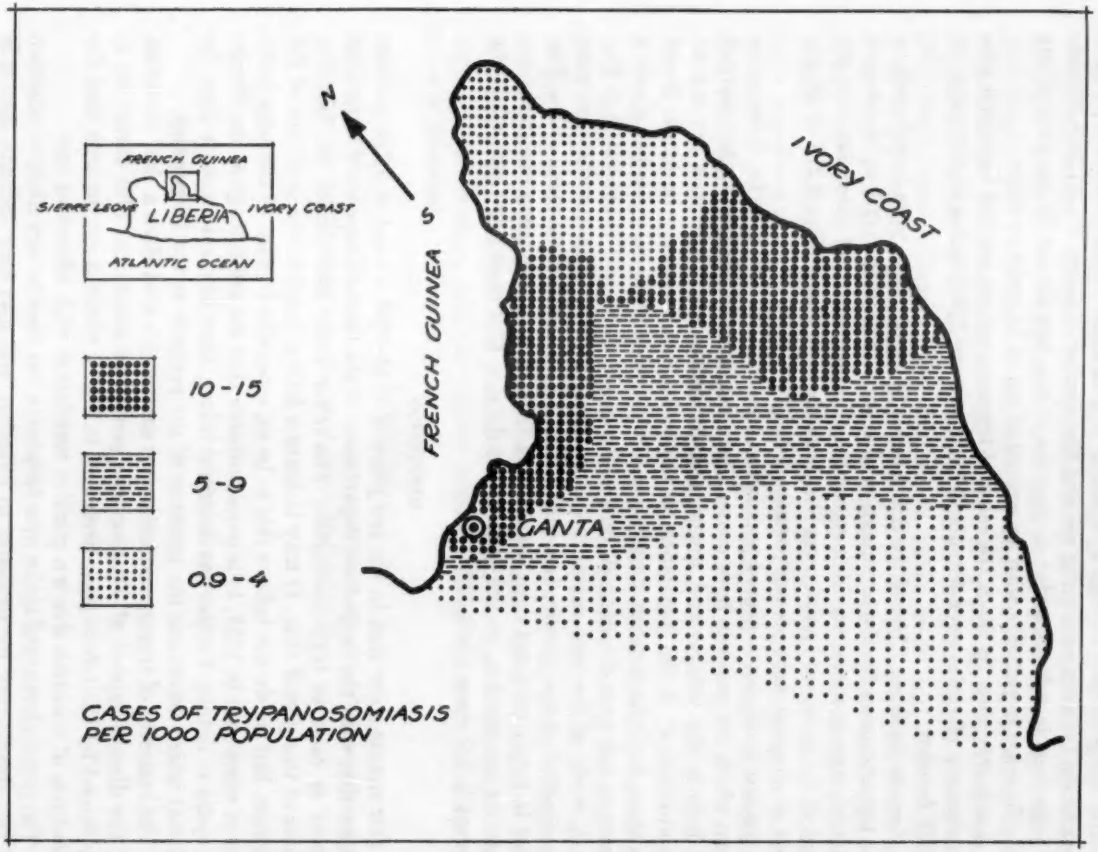

FIG. 3. Map showing geographic distribution of trypanosomiasis cases attending the Ganta Dispensary, Liberia, 1944–1953, and the number of patients from each area, calculated as a yearly average per 1,000 of population.

mated by the examining physician. On the basis of this admittedly arbitrary information, the patients have been classified according to age groups and the results are presented in Fig. 2. A study of this chart shows a striking positive correlation between advancing age and the number of cases. We do have records of infections in infants under six months of age, but almost 80 per cent of our infections are in patients with an estimated age of 15 years or more.

Sex distribution. In the 5,214 cases of trypanosomiasis studied there is a preponderance of males over females. Thus there are 2,901 males as compared to 2,313 females.

Parasite findings. 1,764 of the 5,214 cases, or 34 per cent, were found positive for trypanosomes by the examination of lymph node juice. 1,001 or 19 per cent showed trypanosomes on thick smear blood preparations; of these 449 or 9 per cent of the total were positive on blood examination alone. Thus 2,218 or 43 per cent of all cases were proven parasite-positive.

Disease incidence. A proper population census is not available for those areas from which our patients have come. There are, however, figures for the number of huts in the villages and towns, and by estimating 4.5 persons per hut an approximation of the population in the various sections may be made. Based on these data the number of cases per 1,000 of population calculated as a yearly average, has been determined for the various sections, and is presented in Fig. 3. A study of the map reveals that the area involved does not show an even distribution of the disease, but that patients come from some parts of the District in larger numbers than from others. The highest average annual incidence seen for any section, on the basis of our data, is 13.2 cases per 1,000, while the lowest is 0.9 cases per 1,000.

DISCUSSION

Our results show that in the ten years of 1944–1953 a total of 5,214 patients registering with the outpatient department of the Ganta Dispensary were diagnosed as cases of trypanosomiasis. The fewer cases seen during the last five years of this period (Fig. 1) may indicate a falling trend in the incidence of the disease. But we do not believe this to be so, especially in view of the large number of cases seen in 1951. It is more probable that the endemicity of the disease is cyclic in nature. Further, as discussed below, there are factors other than the disease which determine the presence of the patients at the Dispensary.

The majority of trypanosomiasis cases seen in this area show a slow evolution of the disease typical of *Trypanosoma gambiense* infections. This chronicity is confirmed by the information presented in Fig. 2 where it can be seen that the incidence of infection shows a positive correlation with advancing age.

The preponderance of males over females in our cases is very likely a reflection of the opportunity for exposure to infection, and also may indicate that it is easier for males to make the often long journey to the Dispensary.

Trypanosomes were demonstrated in 43 per cent of all cases. As mentioned earlier, the majority of patients reaching the clinic show moderately or far advanced disease. In these it is often difficult to demonstrate the parasites,

especially if the spinal fluid is not examined. The fact that almost one in five patients showed trypanosomes in the blood, indicates that the examination of a thick blood smear is a valuable diagnostic aid in our group of patients.

Patients seeking treatment at the Ganta Dispensary should be thought of as a sample rather than an adequate index of the number of sick people in a village. And undoubtedly for every case of trypanosomiasis seen at the Dispensary there is at least one case remaining behind. Further, their coming to the Dispensary depends on a variety of factors including fad and fashion, distance from the Dispensary, accessibility and the presence of motor roads, and the economic prosperity prevailing during any given year. Thus our figures are by no means a true index of the amount of trypanosomiasis present in any area. Nevertheless we believe that our results at least give an indication of the relative density of the disease in the different sections, and probably indicate where the more important areas of infection are centered. There would appear to be no question of the importance of trypanosomiasis as a human disease in the Sanoquelle District.

SUMMARY

A total of 5,214 patients from the Sanoquelle District of Liberia, registering with the outpatient department of the Ganta Dispensary during the 10 years 1944–1953, were treated for trypanosomiasis. This number represents roughly 10 per cent of all patients visiting the Dispensary during this period. Trypanosomes were found in the lymph node juice, the blood, or in both, in 43 per cent of the patients. Information is presented on the age and sex distribution of the trypanosomiasis cases, and the relative incidence of infection in the different areas is discussed.

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EXPERIMENTAL INOCULATION OF BACTERIA-FREE *TRICHOMONAS VAGINALIS* INTO HUMAN VAGINAE AND ITS EFFECT ON THE GLYCOGEN CONTENT OF VAGINAL EPITHELIA

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Though many investigators have discussed for long years the pathogenicity of *Trichomonas vaginalis*, no reliable conclusion was reached until a few years ago. Three different opinions have been advanced: 1) the vaginal trichomonad is harmless; 2) relatively pathogenic; or 3) definitely pathogenic. After the development of bacteria-free cultivation of this organism, the controversy may be considered as virtually settled. Most of the cases of experimental inoculation with the bacteria-free strains have revealed definite pathogenicity (Trussell, 1947). However, the mechanism of the symptomatology in vaginal trichomoniasis has not been clarified.

It is said that the glycogen of the vaginal mucosa is significant to the maintenance of the physiological condition of the vagina, since it decreases in cases of microbial infection of the vagina. The present paper refers to the results of experimental inoculation of bacteria-free *T. vaginalis* into the human vagina, and to changes in concentrations of glycogen in the vaginal mucosa following the inoculation.

The authors are greatly indebted to Prof. H. Matsubayashi of the Parasitology Department of Keio University and to Dr. Kinoshita, head of the obstetrical clinic of Saiseikai Chuo Hospital, for advice and aid throughout this study.

MATERIALS AND METHODS

A strain of bacteria-free *Trichomonas* isolated by Asami (1952) was used in the present experiment. The culture medium of this organism has the following formula: the basal medium contains meat extract (1 gm.), peptone (1 gm.), cystein HCl (0.1 gm.), dextrose (0.5 gm.) and 0.1% of methylene blue solution (0.2 ml.) in 100 ml. of distilled water. Human serum (20%) and adequate concentrations of penicillin are added to the basic medium just before use. Without dextrose, the medium does not support growth very well and sometimes the trichomonads disappear on transference.

A two- to four-day-old culture, which was generally in maximum population, was implanted directly into the human vagina in 0.5 ml. amounts, without counting the number of trichomonads present. It was supposed, however, that approximately 250,000 to 750,000 organisms were contained in the inoculum, on the basis of a previous experiment (Asami, 1952).

Nine volunteers, who were admitted to the obstetrical clinic of Saiseikai Chuo Hospital were inoculated. None of these women presented trichomonads as shown by repeated examinations before the experiment, and they were kept away from all possible opportunities of infection by this organism after they

TABLE 1

Description of the patients used in this experiment

Case	Disease or Operation Performed	Menstrual Phase in Inoculation	Age
1	Retrodeviatio uteri	Early proliferative phase	37
2	Myoma uteri	Secretory phase	43
3	Hysterectomia totalis	Obscured by irregular bleeding	42
4	Hysterectomia	Obscured	48
5	Abortion and oophorectomy	10th day after therapeutic abortion	30
6	Abortion	3rd day after therapeutic abortion	32
7	Curettage	4th day after operation	25
8	Oophorectomy	Late proliferative phase	31
9	Oophorectomy	3rd day after therapeutic abortion	28

were admitted to the hospital. None of them were pregnant. Information on these patients is given in Table 1.

The implantation was preceded by determination of the pH of the vaginal discharge, of the vaginal flora and of the glycogen content of the vaginal mucosa using the following procedures: pH was determined by the test paper method; the vaginal flora was classified as Grade I, II and III according to Schröder; the glycogen content of the vaginal epithelia was estimated from the glycogen index (GI), determined by scanning the smear preparations stained with Best's carmine, and estimating the percentage of glycogen-positive cells.

The phase of the menstrual cycle of the volunteers at the time of implantation was recorded because the cyclic physiologic changes of the vaginal discharge may be responsible for the infection.

The cultivated organisms were introduced into the posterior fornix of the vagina with a pipette, and the patient was kept in the lithotomy position for a little while. The inoculated patients were examined periodically for *T. vaginalis*, pH, bacterial flora and GI. The presence or absence of the organism was determined in most instances by microscopic examination and, if necessary, also by cultivation.

The vaginitis caused by the inoculation of trichomonads was treated with carbarsone tablets (Sankyo).

RESULTS

Of the nine patients inoculated, seven were successfully infected with the organism and the remaining two were not infected. Findings before and after inoculation are shown in Tables 1 and 2.

In Case 1, a few trichomonads were seen on the second day after inoculation but they were not recovered on the third day or thereafter by microscopic and cultural examinations. Those found on the second day were probably the inoculated ones which remained alive for that period. No pathological changes in pH, bacterial flora, GI and clinical findings occurred. Thus the infection was not established in this case. In Case 4, no trichomonads could be detected throughout

TABLE 2

Comparison of findings in the vaginal fluid before and after the implantation of T. vaginalis

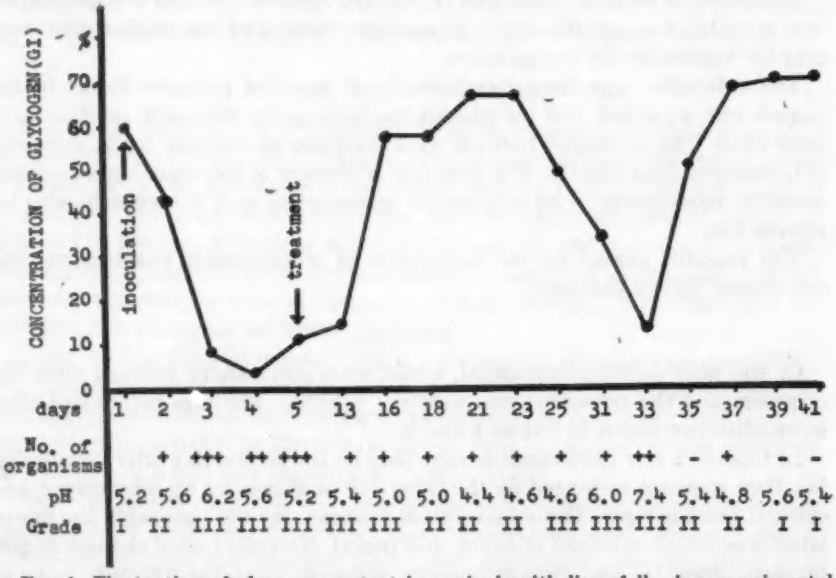
Case	Before Implantation		Incubation Period	After Implantation	
	pH	Grade*		pH	Grade*
1	5.0	I	Failed	5.4	I
2	7.8	II	4 days	6.6	III
3	7.2	II	6 days	5.4	III
4	8.8	II	Failed	7.2	II
5	6.8	II	2 days	5.8	III
6		II	2 days	5.8	III
7	5.0	II	6 days	5.2	III
8	5.2	I	2 days	6.2	III
9	4.8	II	5 days	6.4	III

The pH and Grade after implantation were read on a day indicating maximal population of trichomonads in the vaginae.

* According to Schröder.

the period of observation. A reason for the failure may be that this woman had an extraordinarily abnormal condition of the vagina, with a pH as high as 8.8 and without definite cyclic changes, probably due to previous total resection of the uterus and ovaries (Tables 1 and 2).

In the seven cases successfully infected, trichomonads began to appear in vaginal discharges from the second to the sixth day. The number of the organ-


CONCENTRATION OF GLYCOGEN(GI)
80
70
60
50
40
30
20
10
0
inoculation
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days 1 2 3 4 5 13 16 18 21 23 25 31 33 35 37 39 41
No. of organisms + +++ ++ +++ + + + + + + + ++ - + - -
pH 5.2 5.6 6.2 5.6 5.2 5.4 5.0 5.0 4.4 4.6 4.6 6.0 7.4 5.4 4.8 5.6 5.4
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FIG. 1. Fluctuation of glycogen content in vaginal epithelium following experimental inoculation with *T. vaginalis* (Case 8).

isms increased suddenly three to four days after their first appearance, accompanied by pathological changes of different degree in the vaginal mucosa and discharges. In most of the cases the vaginal discharge was purulent and sometimes frothy, and contained numerous leucocytes. Reddening of the vaginal mucosa occurred in many cases, and frequency of urination and intertrigo of the perineum were noted in two patients.

The GI of the vaginal epithelia began to decrease at an early stage of infection when only a few organisms were detected in the discharge. At the time when numerous trichomonads appeared in discharge, the GI decreased to as low as 2 per cent, while it was 50 per cent or more before implantation. Throughout the course of the infection the GI varied inversely with the number of organisms. The GI generally returned to the level observed before inoculation after treatment and the subsequent disappearance of trichomonads. Fluctuation of GI-value in a representative case (Case 8) is illustrated in Fig. 1. Observations on the morphologic changes of the epithelial cells which appeared in vaginal discharges, seemed to show that superficial epithelial cells decreased and subsuperficial epithelial cells increased with the development of clinical findings. However, more detailed investigations are necessary to draw conclusions on this aspect.

DISCUSSION

Experimental inoculation of vaginal trichomonads into the human vagina have been attempted by several investigators for the purpose of ascertaining their pathogenicity (Matsuda, 1936; Kessel and Gafford, 1940; Stabler, Feo and Rakoff, 1941). A definite pathogenicity has been attributed to the organism since the experimental inoculation of bacteria-free strains into the human vagina was carried out by Trussell and Plass (1940), as cited in the introduction to this paper. The results of the present experiment are in accord with theirs as to the pathogenicity of these parasites. All of the patients successfully implanted developed symptoms or signs of vaginitis of various intensities. It has been considered that abnormal vaginal flora may favor the establishment of the infection, and Case 1, in this experiment, supports the above consideration. Her discharge was Grade I and proved refractory to infection. On the other hand Case 8 was successfully infected though her discharge was also Grade I.

The role of vaginal glycogen in maintaining an acid environment which inhibits the invasion and growth of pathogenic bacteria is well recognized. Ayre *et al.* (1950) observed the relation between GI and vaginal bacteria as influenced by penicillin application. Their experiments indicated that bacteria and leucocytes initially present disappeared and the concentration of glycogen in the vaginal epithelial cells remarkably increased with the use of penicillin. The clinical findings were also greatly improved. In cases of vaginal trichomoniasis, deficiency of glycogen in the vaginal mucosa has been observed by several authors (Löser, 1922; Ishikawa, 1950; Ayre *et al.*, 1950).

The present experiment demonstrated a decrease in the amount of glycogen in vaginal epithelial cells following the implantation of the organism. Since it is

well known that Döderlein bacilli require much glycogen for their multiplication, growth of these bacilli may be inhibited in this condition of the vagina. Thus it is suggested that the vigorous consumption of glycogen by trichomonads facilitates the invasion of the vagina by abnormal bacteria, resulting in vaginitis through synergistic action of trichomonads and bacteria.

SUMMARY

Nine volunteers received bacteria-free *Trichomonas vaginalis* into the vagina. Seven of them became infected and developed vaginitis of varying intensity. The amount of glycogen (GI) in the vaginal mucosa varied inversely with the number of the organisms in the vaginal discharge, with minimal concentrations of 2 per cent in glycogen content in epithelial cells at the period of maximum population of trichomonads. It is suggested that vigorous consumption of glycogen by *Trichomonas vaginalis* facilitates the invasion of bacteria into vagina, with resulting vaginitis.

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EFFECTS OF AN OVERDOSE OF CHLOROQUINE IN A PREGNANT WOMAN

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Research on antimalarial drugs during World War II led to the development of chloroquine: 7-chloro-4-(4-diethylamino-1-methybutylamino)quinoline (SN 7618) which, because of its pronounced suppressive and therapeutic action was adopted as the drug of choice by the U. N. Supreme Command during the recent hostilities in Korea. The Danish hospital ship, Jutlandia, operating under this command, made chloroquine available to the ship's crew. The dosage was a single 0.5 gm. tablet (0.3 gm. base) weekly. Labelled bottles containing the tablets were placed in the dining hall; each member of the crew was directed to take one tablet each Sunday.

CASE REPORT

A member of the medical staff was a female physician, aged 29, 5 months pregnant. Apart from nausea during sailing she had always enjoyed good health. Because of her pregnancy she took vitamin and calcium tablets. These were placed at her table in the dining hall. One Wednesday the patient realized that she had not taken chloroquine the previous Sunday and no calcium for a couple of days. She swallowed one tablet of chloroquine and 5 or 6 tablets from the container labelled "calcium tablets." One hour later she experienced double vision, suffered from severe nausea, and suddenly fainted. She was put to bed, vomited several times, and was very sleepy. Respiration became depressed and difficulty was experienced in breathing and swallowing. Gradually paresthesia and analgesia developed in the skin of the face and neck. It was not possible to find any neurological or ophthalmic disturbances apart from the mentioned symptoms. The diagnosis was uncertain. Poisoning was considered and Chinese food eaten the previous day was suspected, but none of the four other persons participating in that meal showed any symptoms. The chloroquine tablet was blamed too, but the patient had so far tolerated the weekly dose without symptoms. After two or three hours the paresthesia and the analgesia disappeared, respiration became normal, and there was no difficulty in swallowing. Double vision, nausea, and sleepiness persisted for 36 hours.

Five days later, just after dinner, the patient again experienced double vision, dizziness, and nausea, but no other symptoms. During the meal she had taken 2 or 3 tablets from the container labelled "calcium tablets" but then suddenly remembered that the tablets had had an odd taste. An examination of the "calcium tablets" revealed that more than half were chloroquine tablets, evidently mixed by mistake. The following day the patient was well. There was no jaundice and no impairment of kidney function. Laboratory studies were not performed.

Four months later the patient was delivered of a healthy infant. The child is normal in all respects, now three years old.

TOXIC REACTIONS

A perusal of the literature accessible to me revealed no case of death due to prophylaxis or treatment with chloroquine in man. However, several authors have noticed toxic reactions, even with normal doses, but these have all been of milder degree, and only a few symptoms were present in each individual. Observations involved volunteers, soldiers, native populations, and patients suffering from malaria. The complication most often reported was pruritus, which ran to 20 per cent in one study (5). Gastro-intestinal reactions were common too, including anorexia, vomiting, diarrhea, and abdominal pains which in some instances were so severe that further administration was discontinued (10).

Headache is mentioned as a complication, but in some papers (3, 7) dealing with more than 1,000 people there is no mention of headache, while other authors (4, 6, 10) state that upwards of 40 to 50 per cent complained of that symptom. Visual disturbances were mentioned by most of the authors (e.g., 1, 4, 6). These constituted blurring of vision, difficulty of focusing, and double vision.

Difficulty in swallowing and depressed respiration was not reported, nor was paresthesia and analgesia of the skin.

CHLOROQUINE INTAKE DURING PREGNANCY

This problem is discussed by few authors. One states that chloroquine is well tolerated during pregnancy, and another (9) who conducted a field experiment in India, had no complications with "a few" pregnant women who received prophylactic treatment for four months. Berberian and Dennis (2) who directed field trials in the middle East had some experience with chloroquine-intake during pregnancy. They have had 5 cases, of whom three experienced acute attacks of malaria. Each was treated successfully with chloroquine and subsequently maintained on a weekly suppressive regimen for 7 to 14 weeks, respectively. The other two received suppressive chloroquine only for 11 and 14 weeks respectively. All 5 women were delivered of normal infants at term.

NEUROLOGICAL SYMPTOMS IN AMINALS

Many experiments have been performed in animals to determine the amount of drug tolerated per unit body weight. These have included studies in rats and dogs. In the former (8), pathological changes were observed in the liver, kidneys, pancreas, and muscles. In dogs (11) various 4-aminoquinolines were found to induce two types of reactions. One was characterized in the acute phase by vomiting, extreme hyperirritability and convulsions. In its chronic phase, the above symptoms were accompanied by profuse and almost continuous salivation. The other was characterized in its acute phase by a prancing- or rearing-horse reaction. The animal moved about stiff-legged, its neck arched, and its jaw pointed down toward the chest. Respiratory difficulties were prominent and in numerous cases death could be ascribed to respiratory failure. The same reactions were observed in the monkey, except for the prancing-horse type of response. These animals also showed symptoms of visual disturbances indefinable

by objective ophthalmological methods. The chronic phase was characterized by muscle tremors, weakness, and severe depression.

DISCUSSION

Severe toxic reactions in man due to chloroquine have not been described previously. In the case under discussion, severe reactions were definitely manifest but how much chloroquine was ingested it is impossible to say. Initially, the patient swallowed 5 or 6 "calcium tablets" plus one real chloroquine tablet. Since chloroquine tablets were inadvertently mixed with the true calcium tablets, the patient could have ingested as much as 3.5 grams (2.1 grams base) or as little as 1.5 or 2.0 grams (0.9 or 1.2 grams base). Either of the smaller amounts is a fairly heavy dose.

Most drugs produce toxic symptoms in certain individuals. Chloroquine is no exception but reported toxicity has, for the most part, been of milder degree and only a few observers refer to neurological complications. Coggeshall, referred to by Sapero (10), points out that despite the mildness of complaints the consistent presence of visual disturbances in his patients might indicate toxicity of central origin. The careful experimental work in dogs and monkeys (11) showed pronounced neurological symptoms, even respiratory difficulties and numerous deaths from respiratory failure, in these animals. This effect has been emphasized in this case where the neurological symptoms were visual disturbances, depressed respiration, difficulty in swallowing, nausea, vertigo, and—not reported before—paresthesia and analgesia of the skin. As regards ingestion of chloroquine during pregnancy, it appears established that it has no abortive effect and results in no harm to the fetus.

SUMMARY

Description of symptoms following poisoning by an overdose of chloroquine in a pregnant woman is presented. Symptoms were double vision, dizziness, sleepiness, nausea, vomiting, depressed respiration, difficulty in swallowing, and paresthesia and analgesia of the skin of the face and neck. Recovery was complete in 36 hours with no adverse effect on the pregnancy.

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STUDIES IN SHIGELLOSIS

I. General Considerations, Locale of Studies, and Methods[1]

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In the past few years, investigation of the problems of diarrheal disease has resulted in important new information. The specific etiology of many of the diarrheal diseases has been made clear, and there has been improvement in recognition and better reporting of these communicable diseases by public health authorities. It has become apparent, with the development of improved bacteriological diagnostic methods, that a major cause of diarrheal disease is the genus *Shigella*. The historical aspects of bacillary dysentery have been fully reviewed by Felsen (1945).

During World War II, as noted by Weil (1947), interest in bacillary dysentery was stimulated by a number of outbreaks among operating forces of Allied and Axis commands. In these outbreaks, the paralyzing effect of dysentery on the personnel of military missions was once more emphasized. (Stone, 1947; Mariott, 1945; Cheever, 1946; Gear, 1944).

In the civilian population, the importance of *Shigella* infections as a cause of morbidity and of mortality, especially in children, has been pointed out by several recent studies. (Felsen, 1945; Cruickshank, 1943, Hardy and Watt, 1944). The many studies of Hardy and Watt, among others, have demonstrated the ubiquity of *Shigella* infection in the population and the frequent occurrence of subclinical or asymptomatic *Shigella* infections. It is now clear that diarrheal diseases caused by *Shigella* may become prevalent or epidemic in any situation where the sanitation standards are lowered, and where human feces can be transferred by fingers, flies, or other means, through food or water, to another susceptible human.

Among the recent advances in the field of shigellosis, the development of effective treatment by sulfonamides (Marshall *et al.*, 1941; Lyon, 1941; Fairley and Boyd, 1943), and by antibiotic agents (Garfinkel *et al.*, 1953) is of prime importance.

The epidemiological concept of shigellosis has been revised, especially by recognition of the common asymptomatic infection with its implications on transmission of the disease. Despite these and other advances in knowledge, a large number of questions remain unanswered. There appears to be some difference in the clinical disease associated with *Shigella*, with severe or fatal cases

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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described as the mode in some groups, and mild cases reported in other groups. Correlation of clinical severity with a *Shigella* type (i.e., high mortality in *Shig. dysenteriae 1* infections) has not been found uniformly, and the opinion has been expressed that the character of the disease may be changing (Weil, 1943). The existence of naturally or artificially induced immunity to infection of man by *Shigella* is another important question not yet satisfactorily answered. The effectiveness of drug prophylaxis, although encouraging reports have appeared, remains inconclusive. In the treatment of diarrheal disease associated with *Shigella*, resistance to the agents has developed in some cases, making improved therapeutic agents of interest. For the prevention of diarrheal disease associated with *Shigella*, it is important to have more effective drugs for the sterilization of carriers, more information on the duration of carrier states, and on the mechanisms of transmission of *Shigella* from cases and carriers to new susceptibles. There has been some speculation on the possibility of a dual etiology of diarrheal disease by a *Shigella* and an associated viral, bacterial, parasitic or other agent. Investigation on all aspects of shigellosis has been retarded by the lack of a satisfactory experimental animal for comparative studies.

Finally, there has been the comment from several workers that the clinical nomenclature of shigellosis is inadequate, and that it should be revised in accordance with newer knowledge of the disease.

In May, 1952, the Conference on Bacillary and Amebic Dysentery of the Division of Medical Sciences, National Research Council, reviewed current problems in the dysenteries, and made recommendations that further studies should be made on the epidemiological, etiological, preventive and therapeutic aspects of bacillary dysentery. The reports which follow are an attempt to provide information on some of these points.

HEALTH ENVIRONMENT

This Unit has been engaged for some years in epidemiological research studies in an Egyptian village, Sindbis, with a population of approximately 5,000. This village had been studied extensively during 1948–51 from a broad epidemiological standpoint by a Rockefeller Foundation-Egyptian Ministry of Public Health project (Weir *et al.*, 1952) and certain basic health data were available. More important, an excellent rapport with the villagers and the cooperation of the Ministry of Public Health made the long term mechanics of the study feasible. The health environment of Sindbis is more or less typical of the rural Egyptian village, and has been described in detail by Weir *et al.* Certain features, which may be unique in the reader's experience, should be made clear for an understanding of factors relevant to the epidemiology of shigellosis in this environment.

Housing. The village is a compact cluster of houses standing in flat fertile fields which are watered by irrigation canals from the Nile. The houses are constructed of mud brick, and are generally of two stories; each has one or more rooms below, with earth floor and a primitive cooking stove fashioned of mud, in which dung cakes are used as fuel. There is little other furniture. Usually

there is a storeroom for maize, the principal grain crop. Frequently a pigeon roost hangs in the common room, and in all cases one room is reserved for the family animals, commonly consisting of a camel, goats, sheep, donkey, cow, or water buffalo (gamoose). The animals are kept in the home for safe-keeping, and to collect the dung for fuel and fertilizer.

Upstairs, one or two sleeping rooms complete the house. Frequently a mud brick bench is built along one side of the room, with a box below for a fire, so that the family can lie together for warmth in winter. Small open holes without glass are placed high in the walls for light and ventilation. No dish washing or hand washing facilities are usually available in the home, these functions being performed in canals or at deep-well troughs.

Disposal of excreta. Some 60 per cent of the homes have been provided with bored hole latrines, open holes which have foot places on either side and may be covered when not in use. The latrine is used by the family when convenient, although excreta are frequently deposited by children and others in the streets, in the irrigation canals, and in the fields. There is very little sense of social taboo with respect to place of defecation, and it is a commonplace to see an adult defecating beside a heavily travelled street. The toilet of the anus after defecation is probably important in the transmission of intestinal disease and especially shigellosis. The general practice among the poorer economic class is to clean the anus after defecation with the hand, using water when available, or a stick or stone when water is not at hand. No paper or other type of tissue is used. The mother sometimes cleans the buttocks of her infant with a rag or with the sleeve of her costume, and the clothes of the child and mother often become grossly soiled with feces. There is no water borne sewage system. Trash and garbage are frequently thrown in the streets.

Water. During the Rockefeller Foundation-Ministry of Health Project of 1948–51, deep wells (60 ft. with Abyssinian pump) were installed in this village, and bathing and clothes-washing centers were installed in the Rural Health Center nearby. The majority of villagers now use drinking water from wells, but some still prefer to drink raw canal water, and most prefer to bathe in canals. Drinking water is kept in the home in large clay jugs, and each person drinks from a common smaller jug. Private shallow wells are installed in 17.1 per cent of homes (Weir *et al.*, 1952).

Food and food hygiene. The principal item of the villager's diet is unleavened bread, made in flat discs from maize, wheat or barley. The native bean provides the chief source of protein in the diet, with infrequent additions of curd cheese, and of gamoose or cows milk which is ordinarily boiled before use. Meat is rarely eaten and is usually cooked. Many vegetables are available to the villager in season, and some, such as carrots, radishes, lettuce and turnips, are eaten uncooked.

The most notable defect of food hygiene is the absolute lack of protection from dirt or flies either in the markets or homes. As mentioned below, during the summer months these insects occur in tremendous numbers, breeding and feeding in human and animal feces, and crawl over food in hordes. Some human fecal

contamination of truck vegetables probably results from the practice of defecation in the fields and the use of human as well as animal feces as fertilizer.

Flies. The housefly, *Musca domestica*, is a prominent feature of Egyptian village life, being prevalent in fantastic numbers from May to October, with some decrease in breeding during the hotter months of July and August. The transmission of fly borne disease may be uniquely affected by the habits of one of the locally prevalent species, *M. domestica sorbens*, whose preference for breeding in human feces, and whose predilection for feeding on human secretions, described by Peffly (1953), leads to the startling appearance of children with eyes, nostrils, and mouth ringed by flies. Children and mothers accept them, and make little or no attempt to brush them away, since they will be replaced by the same or other flies. In this village, the fly prevalence was effectively controlled by insecticides in 1950, and infant mortality dropped from 227/1000 live births in 1949 to 115/1000 in 1950. In 1951, however, the fly population became resistant to chlordane and DDT, and returned to its former level. Infant mortality also again resumed its previous level. A large part of the salvage of infant life during the period of fly control was attributed to control of fly-borne disease (Weir *et al.*, 1952).

Medical facilities. The village of Sindbis is provided with free medical service by a Rural Health Center of the Ministry of Health. This consists of a new clinic building with 24 beds for general medical cases. The Health Center is staffed by a Medical Officer, several nurses, nurse assistants, a sanitarian and a clerk-technician. Equipment and medical supplies are rather limited. The Health Center at Sindbis is responsible for preventive medicine and medical care of Sindbis and four nearby villages, with a total population of approximately 30,000.

Statistics relating to births, deaths and disease prevalence in this village are fragmentary, and only those obtained by the organized epidemiological survey conducted in this village by Weir (1952) are considered at all accurate.

The attitude of the villager toward health appears to be unusual; he assumes a fatalism concerning disease which seems to prevent him from taking simple steps against it. For example, although 95 per cent of the adult males of this village are infected with schistosomiasis, and although the cycle of this parasite could be quite easily broken by simply stopping the pollution of the irrigation canals with infected urine and feces (no animal host is involved), no amount of propaganda and education has convinced the villager that he should change his toilet habits.

Economic status. The Egyptian villager has endured a marginal existence for centuries. In Sindbis, Weir *et al.*, (1952) found that 92 per cent of families had an income less than $28.80 per month, and 65 per cent of families had an income of below $14.40 per month. 61 per cent of families owned no land.

Endemic diseases of importance in the consideration of diarrheal disease. To complete the health environment description of this Egyptian village as it affects these studies, it is necessary to mention some of the many prevalent diseases, which are generally regarded as causes of diarrheal disease. The people of Sindbis are heavily infected with both *S. mansoni* and *S. haematobium*, with consequent prevalence of the common disabling complications of this disease, anemia and hepatolienal fibrosis. The disease is contracted by bathing or working in the ir-

rigation canals, where the snail host, infected by miracidia from eggs in human excreta, is present in great numbers. Although accurate data on annual incidence of typhoid or other *Salmonella* infections are not available, these diseases are known to be extremely prevalent. It was estimated by Weir that the annual incidence of typhoid in Sindbis was 1,200 cases per 100,000 and that the carrier rate was between 4 and 6 per cent of the general population. This high rate of enteric disease is borne out by Floyd's (1953) finding of *Salmonella* in 5 per cent of blood cultures from 2,000 undiagnosed febrile children. Hookworm is a widespread infestation in some areas of Egypt, and was found in 5 per cent of females in Sindbis by Weir *et al.*, (1952). The villager is parasitized with *Endamoeba histolytica* from early life. In a survey of another similar village, Lawless (1953) demonstrated, in six fecal samples over a period of approximately one year, that *E. histolytica* was present in 97 per cent of 100 subjects on at least one occasion. With this heavy prevalence, an unexpectedly low number of cases of amebic dysentery or amebic hepatitis has been reported.

Poliomyelitis is apparently a highly endemic disease of early life in the Egyptian village; Paul (1952) found evidence of previous infection in 75 per cent of children at the age of four years. Juvenile and adult paralytic cases are not common. No estimate of the contribution to diarrheal disease by gastrointestinal components of poliomyelitis in children is possible, since nonparalytic poliomyelitis is not recognized. It is apparent that these subjects, with their optimum opportunities for acquiring transmissible disease from animals, humans, or vectors, become infected with a wide range of viral diseases, whose effect on morbidity, mortality, or in dual relationships with other diseases such as the diarrheal diseases, is at the present time completely unknown.

The role of other bacteria, bacterial toxins, and chemicals in diarrheal disease in this community is not known. No outbreaks of bacterial food infection or food poisoning have been identified recently in this village.

Faulty nutrition may be a factor in the incidence of diarrheal disease. Weir *et al.*, (1952) found gross pellagra in 1 per cent of children in the age group below five years in this village.

It has been known for many years that shigellosis is highly endemic in Egypt (Ali, 1940; Shawki, 1928). The role of *Shigella* in this endemic diarrheal disease has not been extensively studied. In the particular village under study, the incidence of *Shigella* infections in children was previously shown by Floyd (1954) to be extremely high. *Shigella* organisms were recovered from 97 per cent of a group during a year of weekly observations.

DESIGN AND METHODS OF STUDIES

To accomplish the studies which will be described, 300 children between six months and five years of age were selected in Sindbis village, 20 kilometers from Cairo. Children in this age group were selected because of their availability for long term weekly observations, and because of the impression that diarrheal disease was a frequent cause of morbidity and death in children. This sample represented nearly all the children of this age group, as recorded in the Health

Center birth register, and the only factor affecting selection was the willingness of the mother to cooperate.

All children, on admission to the study, were examined by a medical officer, and the history of previous illness, height, and weight were recorded. The children were generally in good health at the beginning of the project in April, 1953, except for widespread trachoma, personal insects, and frequent upper respiratory disease. Little diarrheal disease was present at the time of initial examination. The 300 subjects were then divided, by selecting numbers in rotation from the list, into three groups of 100.

The first group of 100 was designated the Control Group. These subjects were used for comparative observations on diarrheal disease with other groups of the project. Each subject was visited weekly by a nurse-technician, at which time the general condition was noted, and the number of stools during the preceding 24 hours was recorded. The temperature was taken, and a stool specimen was collected for parasitological examination. A rectal swab was then taken for *Shigella* isolation and placed in a tube of buffered glycerine-saline for transport to the bacteriology laboratory. If a child appeared ill, the nurse-technician instructed the mother to take him to the physician, who kept the record of illness and prescribed indicated therapy. These subjects were followed in this way for one year.

The second group of 100 subjects were designated the Vaccination Group, and were observed and handled in the same manner as the Control Group except that parasitological examination of stools was not done. These children were given, parenterally, a monovalent *Shig. flexneri 3* vaccine. Observations of this group were continued for six months.

The third group of 100 subjects was designated the Prophylaxis Group. These were further divided by selection of numbers in rotation into three groups, designated the Sulfadiazine, Dihydrostreptomycin, and Oxytetracycline Groups. These children were observed and handled exactly as were the Control Group with the exception that they received, three times weekly, a dose of the prophylactic agent being tested in that group. No parasitological observations were made on the stools of these subjects. Observations in this group were continued for 36 weeks.

When a child in any group became ill, he was treated, usually as an outpatient. The diarrheal diseases were treated with any one of the following drugs: sulfadiazine, sulfaphthalidine, dihydrostreptomycin, chlortetracycline (Aureomycin) or oxytetracycline (Terramycin). In the Prophylaxis Group, a different drug was used than that under test in the subject.

Factors affecting results. Certain factors affecting the mechanics of these studies must be mentioned. Because of a language barrier, it was only possible for the U.S. investigators to get information from the subjects and families through interpreters. The unenlightened public health attitude of the villager also resulted in some known misinformation, even to the Arabic-speaking medical officers; for example, a history taken a second time, might be quite different from the original version. The medical officers were aware that some mothers represented their children to be sick (or brought a sick child not in the study group) in order

to get medicine (which could be sold). A fatalism appeared to develop in the family, when a child became very sick, which made it difficult to locate the child and to make observations. It was virtually impossible to admit a sick child to the hospital. No proctoscopic examinations or autopsies were possible. Mothers sometimes became suspicious of observation procedures, particularly of venipunctures, and it was necessary to drop some subjects on this account. There was some unevenness in the quality of observations in the three nurse-technician teams handling the three groups, which cannot be easily assessed.

It was planned to make observations on the therapeutic effectiveness of the several drugs used in the treatment of diarrheal episodes due to *Shigella*. However, the difficulty in making detailed observations on the sick children made the data on treatment results incomplete, and no comparative study of treatment was possible.

Bacteriological methods. At the laboratory the rectal swabs were streaked onto Shigella-Salmonella agar (Difco) and MacConkey agar (Difco) plates. After 24 hours incubation at 37°C., the plates were examined and suspicious non-lactose-fermenting colonies transferred to triple sugar-iron agar (Difco) slants. Cultures that produced reactions on this media typical of *Shigella* were then tested for their biochemical properties. Final identification was made by serological methods.

Parasitological methods. Parasitological examination of the stool was performed by the Merthiolate-Iodine-Formalin technic of Sapero and Lawless (1952) and a modification of that technic for the identification of heavy ova (Blagg, 1954).

DEFINITIONS

In assembling data relating to diarrheal disease and especially shigellosis in these studies, it became necessary to make certain arbitrary definitions.

A *diarrheal episode* was defined as five or more stools on one or more days, and its onset and end were defined by one week of normal stool habit before or after the episode. A diarrheal episode was considered to be associated with a *Shigella* isolation when both occurred not more than one week apart. When a diarrheal episode was ascribed to one *Shigella* type, additional *Shigella* types isolated during the episode were not considered as causal for that episode.

A *Shigella infection* was recorded when a *Shigella* type was isolated, and it was considered persistent when the same type was isolated from the subject thereafter.

An *asymptomatic Shigella infection* was defined as an isolation of a *Shigella* type from an apparently healthy subject, with no record or history of diarrheal disease within a week before or after the isolation.

More detailed descriptions of methods will be described in the individual project reports which follow.

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STUDIES IN SHIGELLOSIS

II. Observations on Incidence and Etiology of Diarrheal Disease in Egyptian Village Children[1]

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Egypt suffers from very high infant and child mortality. In 1949, the Ministry of Finance and Economy reported 112,641 (13.4 per cent) infant deaths in 836,516 live births in Egyptian localities having health bureaus. Of 64,914 infant deaths in which a cause was recorded, over 50 per cent (35,083) were ascribed to "diarrhea and enteritis". Few studies have been made on the etiology of this diarrheal disease (El Gholmy, 1941; Sabri, Abboud and Ali, 1937; Shawki, 1928).

In Sindbis, the village under study, a similarly high infant mortality had been observed. During a health survey by the Egyptian Ministry of Public Health and the Rockefeller Foundation (1948–1951), the infant mortality rate was found to be 245 per 1000 live births (Weir *et al.*, 1952). A large portion of this mortality was attributed to diarrheal disease. With the exception of a preliminary survey in this village, in which it was found that 97 per cent of a group of children had at least one *Shigella* infection during a year of observation (Floyd, 1954), little was known about the etiology of the disease.

The present study was undertaken to determine more exactly the incidence and etiology of diarrheal disease among a group of children in a typical Egyptian village.

METHODS

One hundred children, designated as a "Control Group", were selected as described earlier from 300 subjects concerned in these projects. They were studied for a period of one year, with weekly clinical, bacteriological and parasitological observations as outlined in Section I. The children received, once weekly, a tablet containing 5,000 USP units of vitamin A, 400 USP units of vitamin D, 75 mgm. ascorbic acid, 2 mgm. thiamine HCl, 3 mgm. riboflavin and 20 mgm. nicotinamide. These vitamins were given to this group to duplicate and control the procedure of another group under prophylaxis with antibacterial agents. The children were referred when ill to the medical officer, who began appropriate study and therapy.

RESULTS

At the end of the one year study the group had diminished to 82 subjects, 43 males and 39 females, whose mean age was 28.7 months. Eight children had died. Five died of diarrheal disease of undetermined origin; one died of measles

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

TABLE 1

Frequency of diarrheal episodes among 82 subjects

NUMBER EPISODES	SUBJECTS	
	NUMBER	PER CENT
0	5	6.1
1	9	10.9
2	23	28.0
3	17	20.7
4	17	20.7
5	6	7.3
6	4	4.9
7	1	1.2

TABLE 2

Incidence of diarrheal disease by age groups

SUBJECTS		DIARRHEAL EPISODES	
AGE	No. SUBJECTS	NUMBER	AVERAGE
0 – 12 mo.	13	48	3.6
13 – 24 "	24	74	3.0
25 – 36 "	16	42	2.6
37 – 48 "	22	59	2.6
49 and over	7	12	1.7
TOTAL	82	235	2.8

TABLE 3

Frequency of diarrheal episodes associated with Shigella

NUMBER EPISODES	SUBJECTS	
	No.	PER CENT
0	22	26.8
1	42	51.2
2	14	17.0
3	3	3.6
4	1	1.2

and diarrheal disease; one died of pneumonia; and one of hepatosplenomegaly and ascites. Several of the fatalities due to diarrheal disease had shown a *Shigella* in the stool some weeks before death, but in view of apparent good health at that time, or because of subsequent negative examinations for *Shigella*, none of the deaths was attributed to infection with these organisms.

Eighty seven per cent of scheduled stool collections and 98.3 per cent of scheduled rectal swab specimens were obtained for examination.

Incidence of diarrheal disease. There were 235 episodes of diarrhea among the 82 children, an average of 2.8 per subject per year. Only five children were free from diarrheal disease during the entire period of observation. The frequency of attacks is shown in Table 1. The majority of subjects suffered two, three, or

four episodes of diarrheal disease. The incidence of diarrheal episodes by age groups (age taken at beginning of study) is shown in Table 2. The highest incidence was seen in those less than 24 months old, and decreased progressively with age.

Relationship of diarrheal disease with Shigella. Sixty children of the group suffered 83 episodes of diarrheal disease associated with *Shigella*, with an average of 1.4 such episodes per child. The frequency of attacks of diarrheal disease associated with *Shigella* is shown in Table 3. The largest number of children suffered one attack, and relatively few suffered a third or fourth attack.

With respect to repeated infections by the same *Shigella* type, there were three subjects who showed a second diarrheal episode associated with *S. sonnei* at least eight weeks after the first episode, and one subject showed a second diarrheal episode associated with *S. dysenteriae 2* 11 weeks after the first episode. No instances of three or more diarrheal episodes associated with the same *Shigella* type were noted.

Of the total of 235 diarrheal episodes observed, 35 per cent were associated with the presence of *Shigella*, as shown in Fig. 1. The remaining 65 per cent were of undetermined etiology. There were a total of 168 separate *Shigella* infections, an average of two per subject per year. Approximately one-half are seen, in Fig. 1, to have been associated with diarrheal disease, and one-half were asymptomatic with respect to diarrhea.

In Fig. 2 may be seen the close correlation between the prevalence of diarrheal disease and of diarrheal disease associated with *Shigella* infections. This figure also shows the seasonal incidence of total diarrheal disease and of *Shigella* diarrhea and dysentery among the children under study. The incidence curve during 1952–3 was rather unusual in that, in addition to the two peaks ordinarily observed during spring and late summer (Ali, 1940), a third peak was noted during November and December.

Relationship of diarrheal disease with E. histolytica. Of 3,764 stool specimens examined, 578 (15%) showed *E. histolytica*. These protozoa were demonstrated at least once in the stools of 83 per cent of the subjects. The relationship of diarrheal disease with the presence of *E. histolytica* is shown in Fig. 3, with a

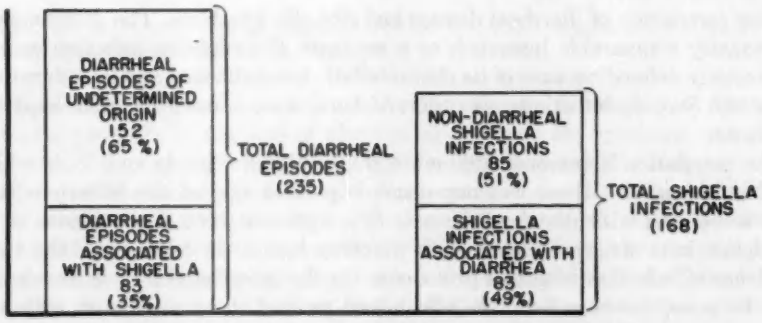
DIARRHEAL EPISODES OF UNDETERMINED ORIGIN 152 (65%)
DIARRHEAL EPISODES ASSOCIATED WITH SHIGELLA 83 (35%)
TOTAL DIARRHEAL EPISODES (235)
NON-DIARRHEAL SHIGELLA INFECTIONS 85 (51%)
SHIGELLA INFECTIONS ASSOCIATED WITH DIARRHEA 83 (49%)
TOTAL SHIGELLA INFECTIONS (168)

FIG. 1. The relationship of *Shigella* infections and diarrheal disease.

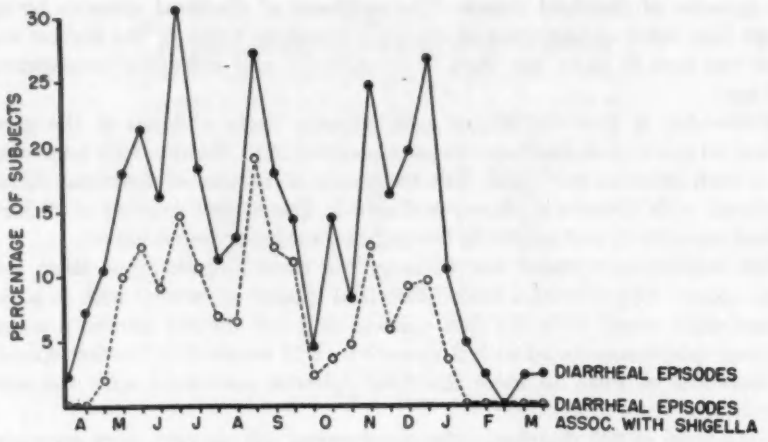

FIG. 2. Incidence of total diarrheal episodes and diarrheal episodes associated with *Shigella* by bi-monthly periods.

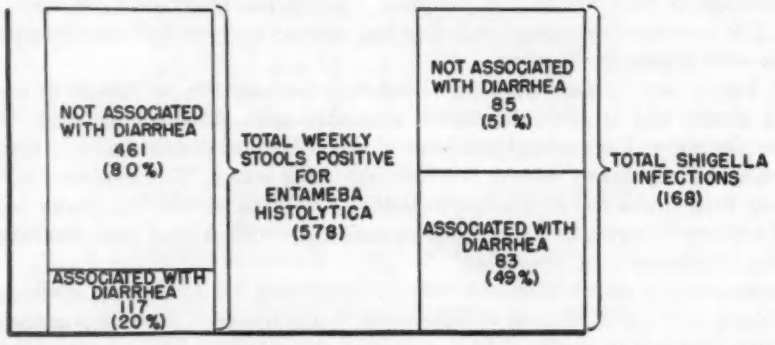
NOT ASSOCIATED WITH DIARRHEA 461 (80%)
ASSOCIATED WITH DIARRHEA 117 (20%)
TOTAL WEEKLY STOOLS POSITIVE FOR ENTAMEBA HISTOLYTICA (578)
NOT ASSOCIATED WITH DIARRHEA 85 (51%)
ASSOCIATED WITH DIARRHEA 83 (49%)
TOTAL SHIGELLA INFECTIONS (168)

FIG. 3. Relationship of *Endameba histolytica* and *Shigella* with presence of diarrheal disease.

similar correlation of diarrheal disease and *Shigella* infections. The diagrams are not exactly comparable inasmuch as a separate *E. histolytica* infection cannot be precisely defined because of its characteristic intermittency and long duration, while the *Shigella* infections, as observed here, were considered to be separate incidents.

The correlation is demonstrated more graphically in Figs. 4a and 4b, in which total infection with these two organisms is plotted against the infections that were associated with diarrheal disease. It is apparent that the incidence of *E. histolytica* infections associated with diarrhea had little relation to the total incidence of infection with this protozoan. On the other hand a close correlation may be noted between total *Shigella* infections and those associated with episodes of diarrheal disease.

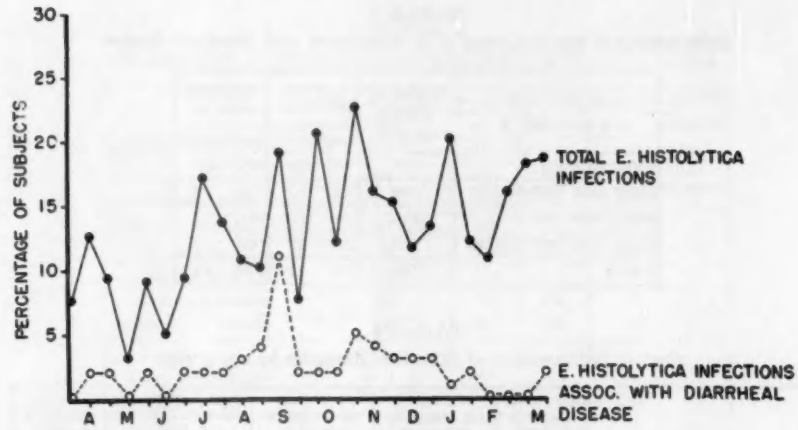

FIG. 4A. Incidence of total *E. histolytica* infection and *E. histolytica* infection associated with diarrheal disease by bi-monthly periods.

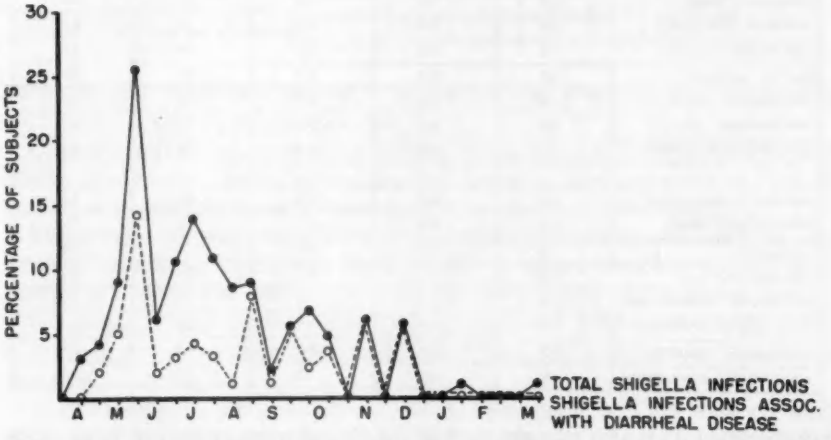

FIG. 4B. Incidence of total *Shigella* infection and *Shigella* infection associated with diarrheal disease by bi-monthly periods.

The data in Table 4 show the relationship of various forms of *E. histolytica* with the presence or absence of diarrhea at the time the specimen was taken. The total number of positive stools, 782, represent many in which more than one form of *E. histolytica* was found in the same specimen. There is no significant difference in the association of diarrhea with the presence of trophozoites as compared with cystic forms, nor with "small race" forms as compared with "large race" forms. Although diarrhea was frequently observed at the time of isolation of *E. histolytica*, no large trophozoites with ingested red cells, which have been considered typical findings in amebic dysentery, were observed in

TABLE 4

Relationship of various forms of E. histolytica with diarrheal disease

FORM OF E. HISTOLYTICA	NUMBER OF STOOLS POSITIVE	DIARRHEA PRESENT		DIARRHEA ABSENT	
		No.	PERCENT	No.	PERCENT
LARGE RACE CYSTS	224	40	18	184	82
LARGE RACE TROPHOZOITES	102	24	24	78	76
SMALL RACE CYSTS	315	74	23	241	77
SMALL RACE TROPHOZOITES	141	38	27	103	73
TOTAL	782	176	22	606	78

TABLE 5

Cumulative incidence of intestinal parasites by age groups

	UNDER 1 YEAR (13 SUBJECTS) PER CENT INFECTED	1–2 YEARS (25 SUBJECTS) PER CENT INFECTED	2–3 YEARS (16 SUBJECTS) PER CENT INFECTED	OVER 3 YEARS (28 SUBJECTS) PER CENT INFECTED	TOTAL GROUP (82 SUBJECTS) PER CENT INFECTED
ENTAMEBA HISTOLYTICA	54	80	94	93	83
ENTAMEBA COLI	69	96	100	100	94
ENDOLIMAX NANA	39	76	100	100	83
IODAMEBA BUETSCHLII	30	56	62	79	61
DIENTAMEBA FRAGILIS	15	12	50	39	29
GIARDIA LAMBLIA	100	100	87	96	96
CHILOMASTIX MESNILI	23	52	87	75	62
TRICHOMONAS HOMINIS	23	28	37	39	32
ENTEROMONAS HOMINIS	7	48	70	64	51
ASCARIS LUMBRICOIDES	84	96	100	100	96
HYMENOLEPIS NANA	54	64	81	92	76
HOOKWORM	15	28	37	50	35
TRICHOSTRONGYLUS	46	24	31	64	41
ENTEROBIUS VERMICULARIS	8	16	25	60	32
HYMENOLEPIS DIMINUTA	0	8	6	25	12
SCHISTOSOMA MANSONI	38	36	44	40	39
SCHISTOSOMA HEMATOBIUM	15	12	12	4	10

any specimen. It is also relevant that in the clinical management of these cases, the medical officers did not consider any case of diarrheal disease to be caused by *E. histolytica*, and did not prescribe anti-amebic therapy in any case.

The relationship of diarrheal disease with other intestinal parasites. The heavy parasitization of the subjects is shown in Table 5. The relationship of each parasite with the presence or absence of diarrhea at the time of observation is shown in Table 6. It is difficult to determine the etiological role in diarrheal disease of any one parasite but it may be noted that similar levels of correlation were found for both accepted pathogens and those usually considered as commensals.

The relationship of diarrheal disease with other diseases. Salmonella typhi was isolated from rectal swabs on three subjects. In view of the limitations of the

TABLE 6

Relationship of certain intestinal parasites with diarrheal disease

	NUMBER OF STOOLS POSITIVE	DIARRHEA PRESENT		DIARRHEA ABSENT	
		No.	PERCENT	No.	PERCENT
ENTAMEBA COLI	839	174	21	665	79
ENDOLIMAX NANA	572	131	23	441	77
IODAMEBA BUETSCHLII	138	25	18	113	82
DIENTAMEBA FRAGILIS	41	10	24	31	76
GIARDIA LAMBLIA	1049	264	25	785	75
CHILOMASTIX MESNILI	149	40	27	109	73
TRICHOMONAS HOMINIS	35	10	29	25	71
ENTEROMONAS HOMINIS	66	20	30	46	70
ASCARIS LUMBRICOIDES	973	229	24	744	76
HYMENOLEPIS NANA	423	114	27	309	73
HOOKWORM	46	11	24	35	76
TRICHOSTRONGYLUS	56	13	23	43	77
ENTEROBIUS VERMICULARIS	49	8	16	41	84
HYMENOLEPIS DIMINUTA	21	3	14	18	86
SCHISTOSOMA MANSONI	36	11	31	25	69
SCHISTOSOMA HEMATOBIUM	12	3	25	9	75

bacteriological methods employed, it is possible that more *Salmonella* infections were present, and were not identified.

Clinical findings indicated no important causal role for staphylococci or their toxins, streptococci, or for other agents of bacterial food poisoning or food infection. No chemical cause of diarrhea was suspected or identified.

There was a frequent association of respiratory symptoms with diarrhea, and in some instances *Shigella* was found in this symptom-complex. Several small groups of cases of measles occurred among the subjects in the winter months.

No attempts were made to identify the presence of viral agents in the stools. It is possible that some part of the diarrheal disease of undetermined origin was due to viral agents.

Nutritional disease was not prominent in these subjects. They were generally well nourished. As noted previously, they received maintenance amounts of vitamines during the study. No frank avitaminoses were recognized.

Clinical aspects of diarrheal disease. The clinical episodes of diarrheal disease observed in this group were, in general, mild. Classical dysentery, with blood and mucopus in the stool, was rare. Those cases associated with *Shigella* did not appear, on the average, different in severity or in duration from the cases of undetermined etiology.

In Fig. 5, the clinical severity is compared with *Shigella* type in 186 separate *Shigella* infections, including those of subjects who were dropped from the study, or who died. Only those *Shigella* types which were isolated five or more times are shown. The highest percentage of severe infections and the lowest percentage of non-diarrheal infections were associated with *S. dysenteriae 1*. While other types, such as *S. dysenteriae 2*, *S. dysenteriae 3–7*, *S. flexneri 6* and *S. sonnei*,

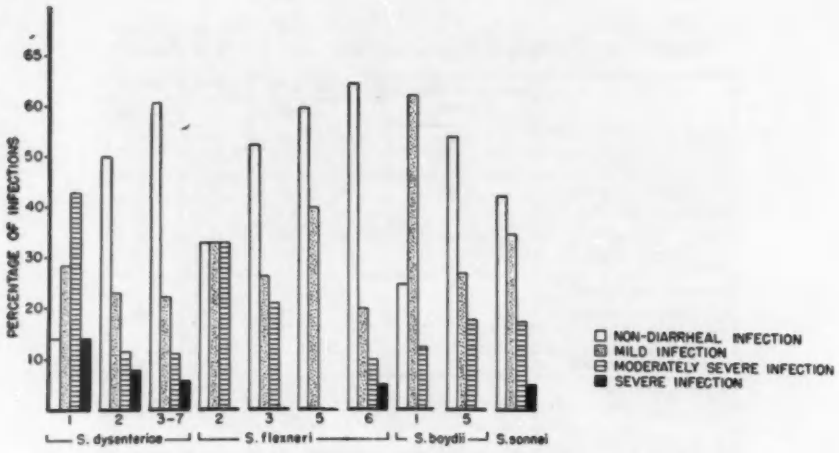

FIG. 5. Relationship of *Shigella* type with clinical severity. 186 cases.

were also associated with severe infections, these organisms were isolated from many asymptomatic cases.

The cases suffering from diarrheal disease were treated with sulfadiazine, sulfaguanidine, sulfaphthalidine, streptomycin, chlortetracycline (Aureomycin) or oxytetracycline (Terramycin). It was the impression of the medical officers that all these drugs were effective; however, treatment data were not sufficiently complete to warrant a detailed analysis.

No sequelae of *Shigella* dysentery such as arthritis or uveitis were noted. No instances of chronic colitis were clinically identified, although proctoscopy was not done. No atypical clinical forms of shigellosis were recognized.

DISCUSSION

The endemicity of diarrheal disease in this group of children offered an opportunity for a systematic long term etiological study. From the data presented, it is apparent that *Shigella* infection is a major etiological factor in the diarrheal disease of this community. While the role of other bacteria, viruses, toxins, chemical agents, nutritional disease, or intestinal parasites was not precisely determinable, no major role was ascribed to these possible causal agents. In view of the close correlation between the total incidence of diarrheal episodes and those in which *Shigella* were isolated through the year, it is likely that many more cases of diarrhea were due to *Shigella* than were so identified. This view is strengthened by the known failure of the bacteriological methods to isolate *Shigella*, when present, in all cases. It is probably incorrect to regard *Endamoeba histolytica* as a commensal in all cases of diarrheal disease in which it was associated in these subjects. However, the fact that no case was considered on clinical grounds to require anti-amebic therapy, the high rate of *E. histolytica* infection with, as well as without, diarrhea, and the lack of correlation between

the prevalence of *E. histolytica* and diarrheal disease through the year, would appear to indicate that *E. histolytica* was not a significant cause of diarrheal disease. Similarly, other intestinal parasites, including several which are generally considered as etiological agents in diarrheal disease, were not ascribed an important causative role in these subjects. In particular, diarrheal disease was not recognized as due to *Schistosoma haematobium* or *mansoni*, *Ascaris lumbricoides*, or *Giardia lamblia*, even when these were repeatedly present in the stools.

A comment may be made on the controversial subject of clinical immunity following an attack of *Shigella* diarrhea or dysentery. It has been the opinion of many observers that "conditioning", or some degree of clinical immunity, occurs with continued exposure to *Shigella* infection. This immunity has been thought to be of short duration (Smith *et al.*, 1952), and severe *Shigella* dysentery has often been observed among those who have resided for long periods in a highly endemic shigellosis environment. The high prevalence of diarrheal disease associated with *Shigella* in these subjects during the peak incidence of disease, and the instances of second attacks of disease with the same *Shigella* type in these subjects, would imply that no firm or long lasting immunity developed. On the other hand, the five children who did not suffer diarrheal disease, and who are likely to have been exposed to *Shigella*, may suggest the development of immunity in these instances. On theoretical grounds, immunity to one type of *Shigella* might be of little value in protection against other serological types.

The high incidence of *Shigella* infections with no diarrheal symptoms has been emphasized by Hardy and Watt (1944) and others, and was noteworthy in this study. No data are available, in terms of local or systemic immunity or of virulence of *Shigella*, which satisfactorily explain this presence of *Shigella* in the apparently healthy human gut. It is possible that some of these cases were carriers following an unreported attack of the disease ("convalescent carriers"). Others, from whom the same organisms were isolated upon several occasions, might be considered as carriers following an asymptomatic infection ("passive carriers"). However, there remain a large number of cases from whom a particular *Shigella* type was isolated only once during the year. It may be presumed in these instances that the *Shigella* were merely ingested and excreted, without an infection resulting in the intestine of the subject.

The clinical description of the various known forms of *Shigella* infection is hampered by lack of appropriate clinical terms, and improvement in the clinical nomenclature of shigellosis has been proposed (Hardy, 1944; D'Antoni, 1943). The use of such etiological clinical terms as *Shigella* dysentery, *Shigella* diarrhea, *Shigella* carriers, and the like, would be preferable to the inadequate terms now in use, such as "bacillary dysentery", "enteritis", or "summer diarrhea", or to locality names, such as "Shanghai belly" and "Gyppy tummy".

Finally, it should be stated that the subjects reported here are somewhat unique. The high endemicity of shigellosis and low state of environmental sanitation in this fixed community of children may present a different shigellosis situation from other communities and ages. Certainly the diarrheal disease observed here is quite different in severity from that seen, for example, in field

troops or in prisoner of war camps, as recently reported by Garfinkel *et al.*, 1953. The factors which produce mild forms of shigellosis in one community, and severe or fatal *Shigella* dysentery in another situation, can only be subjects for speculation in the light of present knowledge.

SUMMARY

The incidence and etiological factors of diarrheal disease in 100 Egyptian village children were observed for one year. There were on the average 2.8 separate episodes of diarrhea per subject. Five children suffered no attacks. Thirty-five per cent of the diarrheal episodes were associated with *Shigella* isolation, while *E. histolytica*, although highly prevalent, did not show a close correlation with diarrheal disease incidence. This was also true for other intestinal parasites. Evidence is presented which indicates lack of firm or long-lasting clinical immunity to diarrheal disease caused by *Shigella*. A revision of clinical nomenclature in shigellosis is needed.

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STUDIES IN SHIGELLOSIS

III. A Controlled Evaluation of a Monovalent *Shigella* Vaccine in a Highly Endemic Environment[1, 2]

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Active immunization against shigellosis has been the subject of a considerable amount of research for a number of years. The literature, as reviewed by Weil (1947), is in general agreement that vaccines made from *Shigella* organisms are antigenic, producing significant levels of agglutinating and mouse protecting antibodies when inoculated into man and laboratory animals. However, it long has been controversial whether such immunization in humans results in actual increased protection against *Shigella* infection.

It has been reported that monovalent and polyvalent *Shigella* vaccines, introduced orally or parenterally, reduced morbidity in populations exposed to infection (Caldwell and Hardwick, 1943; Dolman, 1948; Johns and Chalk, 1943; Klimentova, 1945; Munro, 1930; Paddle, 1938; Ratner, 1949; Scott, 1938; Shumkin, 1950; Vincent, 1921; Wilkens and Wells, 1924). On the other hand, the studies of Hardy, DeCapito and Halbert (1948), Shaughnessy *et al.* (1946), and Thale and Opper (1948) failed to demonstrate clinical protection against shigellosis by vaccination.

The situation with respect to present knowledge concerning the protective value of *Shigella* vaccines was well summarized by Weil (1947): ".... definite proof of the value of active immunization can only be obtained by exposure of an immunized population under adequately controlled conditions. Data on this crucial point have not been forthcoming in a way to eliminate reasonable doubt."

Thus it appeared that a further trial of a *Shigella* vaccine in man, adequately controlled and with challenge by the naturally occurring disease in endemic form, would provide additional and valuable information on this subject. The high endemicity of shigellosis in Egyptian children in the village of Sindbis throughout one-half of the year, as determined by studies during the previous year (Floyd, 1954), made this environment an ideal site for such an evaluation study. In March, 1953, with the cooperation of the Egyptian Ministry of Public Health, an experiment was begun.

MATERIALS AND METHODS

A. *Vaccine.* From the studies on shigellosis in this village (cited above) it had been observed that at least 5 different *Shigella* species or types were probably

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[2] Appreciation is expressed to Commander L. A. Barnes (Medical Service Corps) USN, U.S. Naval Medical Research Institute, Bethesda, Md., for advice and assistance in planning and inaugurating this study.

of major etiological importance. It was recognized that in an evaluation of the efficacy of vaccination against shigellosis in this environment the vaccine ideally should contain most, if not all, of these types of organisms. The choice of a vaccine was governed by the availability of one that had been thoroughly tested in humans for antigenicity and toxicity. A polyvalent preparation that met both specifications was not readily obtainable and a tested monovalent *Shig. Flexneri 3* vaccine was available. Since the study of the previous year had shown that 40 per cent of a group of children from the same village as those chosen for this study had had at least one *Shig. flexneri 3* infection during a period of one year, it was felt that an adequate challenge was assured.

The vaccine used was prepared at the U. S. Army Medical Service Graduate School, Washington, D. C., after the method of Cooper and Keller (1948). It was heat-killed and standardized to contain 2.5 billion organisms per ml. and 9.2 mgm. nitrogen per 100 ml., and was preserved with 1:10,000 merthiolate. In doses as high as 1.0 ml. it had not produced severe local or systemic reactions in adolescents or adults (Cooper *et al.*, 1948; Barnes *et al.*, 1949), and had been shown capable of eliciting significantly high and long lasting humeral antibody levels in humans (Bennett *et al.*, 1949; Barnes *et al.*, 1951; Cooper *et al.*, 1948).

Since a total dose of 2.0 ml. of vaccine had been used with safety and had produced measurable antibody responses in the young adults in the field trial of Barnes and co-workers, it was felt that one-half this amount would be adequate for the infants and young children of the present study. To determine the toxicity of this planned dosage, 12 two-year-old children, not in the study, were divided into three groups of four subjects each. The groups were vaccinated subcutaneously with 0.5 ml., 0.35 ml., and 0.2 ml., respectively. During three days of observation none of the children experienced reactions more severe than those encountered with a typhoid-paratyphoid vaccine. On the basis of this titration it was decided to give the total dose of 1.0 ml. in three divided amounts of 0.3 ml., 0.3 ml., and 0.4 ml. subcutaneously at weekly intervals.

B. *Selection of vaccination and control groups.* As described in Section I, 300 children had been selected in a village near Cairo for the studies on shigellosis. These children were divided into three equal groups by alternate selection from a muster roll; the first hundred were designated as the "Control Group", the second hundred as the "Vaccinated Group" and the third hundred were used in another phase of the project.

The children in the vaccinated and control groups were between the ages of one month and five years, with means of 27.5 and 28.5 months respectively. The division of sexes was almost even in both groups (Table 1).

C. *Methods of observation.* Beginning the last week in March, 1953, and continuing through October, 1953, the children in both groups were visited weekly by nurse-technician teams. During these visits a rectal swab culture was obtained, the rectal temperature taken, the mother questioned concerning the bowel movements of the child during the past week, and the general condition of the child noted. Obviously ill children were directed to the Rural Health Center for diagnosis and treatment. Children with diarrheal disease were visited

TABLE 1

Age and sex data on control and vaccinated groups

Group	Number Subjects	Sex		Mean Age Months
		M	F	
Control	100	51	49	28.5
Vaccinated	100	54	46	27.5

daily, as far as possible, throughout their illnesses. Rectal swabs were carried to the laboratory in sterile tubes containing 2 ml. of glycerine-saline solution. At the laboratory, identification of *Shigella* was done by the methods described in Section I.

D. *Vaccination.* Studies of the previous year had shown a distinct seasonal incidence of *Shigella* infections with the season of greatest prevalence occurring in late spring and early summer. In order that the vaccinated children should have the fullest possible protection from the vaccine, and at the same time to insure a maximum challenge, vaccination was carried out just prior to the expected seasonal rise in incidence. During the last two weeks in April and the first week in May, all 100 children were vaccinated subcutaneously at weekly intervals with 0.3 ml., 0.3 ml., and 0.4 ml. of the *Shig. flexneri 3* vaccine. Children in the control group were not given a placebo inoculation.

RESULTS

Children in both control and vaccinated groups were observed, by the methods described above, for six months following completion of vaccination. At the end of this period, due to attrition from deaths, non-cooperation, or changes in residence, there were 88 control and 93 vaccinated children remaining in the study.

Effect of vaccination on incidence of Shigella flexneri 3 *infections.* During the period of observation, 18 (20.4 per cent) of the 88 children in the control group and 25 (26.8 per cent) of the 93 vaccinated children were infected with *Shig. flexneri 3* (Table 2). None of the children experienced second attacks with this organism. The proportion between diarrheal and non-diarrheal infections was almost the same in both groups (Table 3).

TABLE 2

Subjects in control and vaccinated groups infected with S. flexneri 3

Group	SUBJECTS		
	Total Number	Infected with S. flexneri 3	
		Number	Percent
Control	88	18	20.4
Vaccinated	93	25	26.8

TABLE 3

Infections with Shigella flexneri 3 in control and vaccinated groups

Group	INFECTIONS				
	Total	Diarrheal		Non-Diarrheal	
		Number	Percent	Number	Percent
Control	18	12	66.6	6	33.3
Vaccinated	25	15	60.0	10	40.0

TABLE 4

Infections with other Shigella in control and vaccinated groups

Group	INFECTIONS				
	Total	Diarrheal		Non-Diarrheal	
		Number	Percent	Number	Percent
Control	143	60	41.9	83	58.1
Vaccinated	142	48	33.8	94	66.2

TABLE 5

Total diarrheal episodes in control and vaccinated groups

Group	DIARRHEAL EPISODES				
	Total	Assoc. with Shigella		Unknown Etiology	
		Number	Percent	Number	Percent
Control	164	72	43.9	92	56.1
Vaccinated	144	63	43.7	81	56.3

Effect of vaccination on incidence of other Shigella *infections.* The numbers of infections with *Shigella* other than *Shig. flexneri 3* are shown in Table 4. There were 143 such infections in the control group and 142 among the vaccinated children. A somewhat higher percentage of infections in the control group were accompanied by diarrhea.

Effect of vaccination on incidence of diarrheal disease. In Table 5 are shown the numbers of diarrheal episodes occurring among the children in both groups. A slightly larger number of episodes occurred in the control group. The proportion of episodes that were associated with *Shigella* was the same in the two groups.

Cumulative percentages of children initially infected with *Shig. flexneri 3* are shown in Figure 1. The rate at which the children in the two groups became infected was comparable, although in the vaccinated group the infections were noted slightly sooner and continued to occur later than in the control group.

Incidence of *Shig. flexneri 3* infections, of other *Shigella* infections and of diarrheal episodes in control and vaccinated groups is shown in Figures 2, 3 and

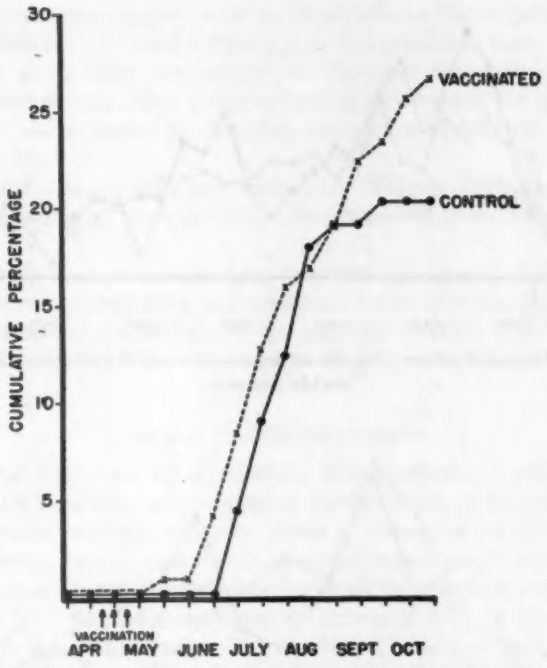

FIG. 1. Cumulative percentage of subjects originally infected with *S. flexneri 3*.

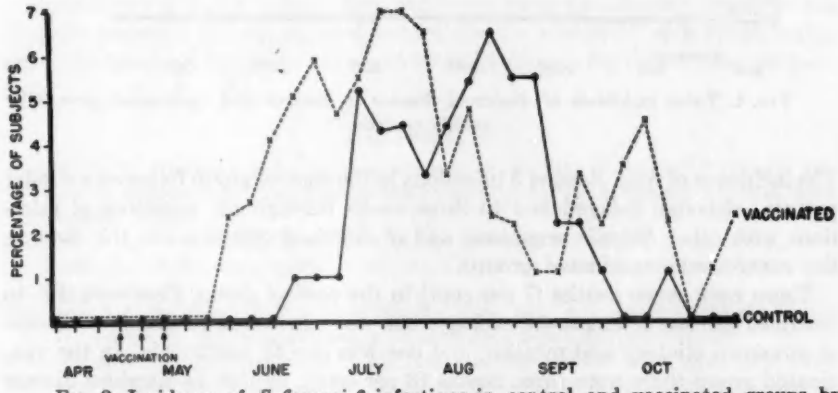

FIG. 2. Incidence of *S flexneri 3* infections in control and vaccinated groups by weekly periods.

4. *Shig. flexneri 3* organisms first were recovered from the children in the vaccinated group three weeks after completion of immunization. The incidence of infection with these organisms in this group rose steadily over the next two months, reaching a peak in July. Thereafter the incidence declined until the latter part of September and early October when a secondary rise was noted.

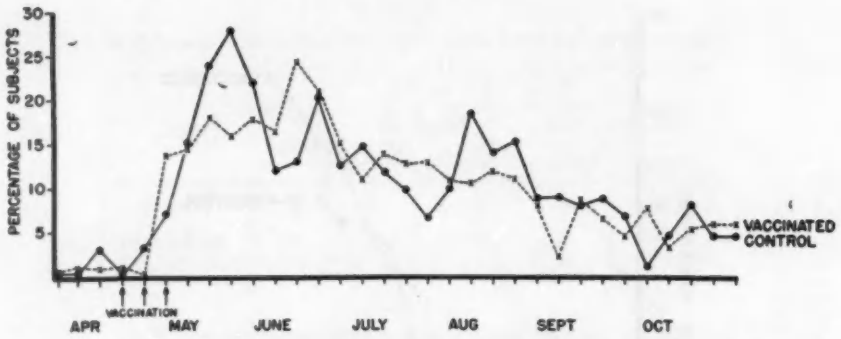

FIG. 3. Incidence of other *Shigella* infections in control and vaccinated groups by weekly periods.

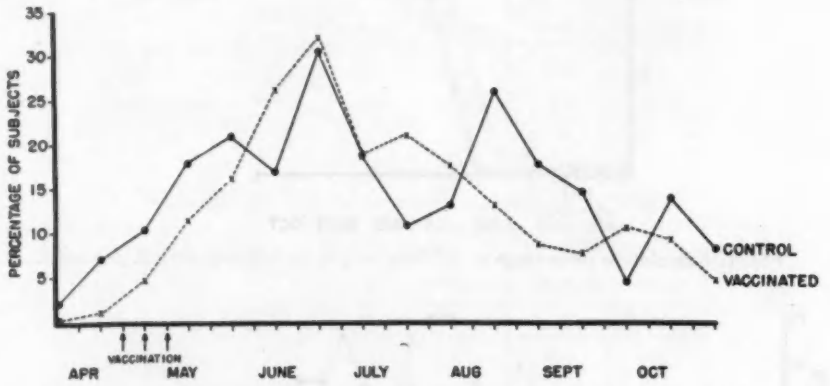

FIG. 4. Total incidence of diarrheal disease in control and vaccinated groups by weekly periods.

The incidence of *Shig. flexneri 3* infections in the control group followed a similar pattern, although delayed two to three weeks throughout. Incidence of infections with other *Shigella* organisms and of diarrheal episodes was the same in the control and vaccinated groups.

There were seven deaths (7 per cent) in the control group. Five were due to diarrheal disease of unknown etiology, one was attributed to diarrheal disease of unknown etiology and measles, and one was due to pneumonia. In the vaccinated group there were three deaths (3 per cent), all due to diarrheal disease of unknown etiology. In several cases of fatal diarrheal disease in both groups, *Shigella* were isolated during the few weeks preceding death, but the relationship was considered not clear enough to establish etiology.

DISCUSSION

From the results presented it is apparent that protection against infection with *Shig. flexneri 3* was not conferred by parenteral immunization with a vac-

cine of the homologous organisms. It happened that a higher percentage of the vaccinated children were found infected with this organism than of the children in the control group who were exposed to the same naturally occurring challenge. There was no indication that vaccination ameliorated the infections since the percentage accompanied by diarrhea was approximately the same in both groups of children.

No protection against infection with other *Shigella* organisms was noted. Comparable numbers of these infections were reported from both groups in the study.

Infections with *Shig. flexneri 3* began to occur in the vaccinated group within three weeks after immunization and continued to occur in numbers comparable to those found among the controls for the next four months. This was the period during which the greatest amount of protection should have been conferred by the vaccine.

SUMMARY AND CONCLUSION

One hundred Egyptian village children were immunized with 1.0 ml. of a *Shigella flexneri 3* vaccine, given in three divided doses of 0.3 ml., 0.3 ml. and 0.4 ml. at weekly intervals. A similar group of unvaccinated children were observed as controls. During a six month observation period following vaccination, 25 (26.8 per cent) of the children who received the vaccine were found to be infected with *Shig. flexneri 3* organisms as compared with 18 (20.4 per cent) of the children in the control group. The number of infections with other *Shigella* organisms, and the number of episodes of diarrheal disease, was approximately the same in both groups of children.

Immunization with a *Shigella flexneri 3* vaccine was not effective in preventing *Shigella flexneri 3* infections, or diarrheal disease associated with these organisms, in a group of Egyptian village children living in a highly endemic environment.

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STUDIES IN SHIGELLOSIS

IV. A Controlled Trial of Sulfadiazine, Dihydrostreptomycin and Oxytetracycline as Long Term Prophylaxis Agents in a Highly Endemic Environment for Shigellosis[1]

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The prevention of diarrheal disease associated with *Shigella* organisms by prophylaxis with a chemotherapeutic or antibiotic agent would be of great importance in the control of outbreaks in civilian groups and in military units. This is particularly true in instances such as disaster in urban communities or in military and naval forces, during operations when sanitation may become temporarily compromised.

With the reported effectiveness of sulfonamide drugs (Marshall *et al.*, 1941; Lyon, 1941; Fairley and Boyd, 1943; Hardy and Cummins, 1943), and, more recently, of streptomycin (Hardy and Halbert, 1948) and of oxytetracycline (Garfinkel *et al.*, 1953) in the treatment of *Shigella* dysentery, it appeared possible that they might also be of value as prophylactic agents.

There have been several reports concerning the prophylactic use of sulfonamides in endemic situations and in localized outbreaks of *Shigella* dysentery. The results of these trials have been somewhat inconclusive. Scott (1943), in an outbreak of Sonne dysentery, administered sulfaguanidine to well contacts and to the nursing staff, with prompt subsidence of the outbreak. Lucchesi and Gildersleeve (1943) used the same drug in a hospital outbreak of dysentery, and no further cases were observed in 45 children receiving sulfaguanidine, although cases developed in another wing of the hospital among children who were not given prophylaxis. Cornell *et al.* (1943) reported that an outbreak of *Shigella* dysentery was controlled and *Shigella* organisms eradicated among 97 members of a military garrison by giving courses of sulfaguanidine to all men who showed positive stool cultures. Hardy and Watt (1944) used sulfonamides prophylactically in heavily infected groups totalling 1,646 persons. There was a rapid disappearance of clinical cases and *Shigella* infections. Cheever (1946) reported the prophylactic use of sulfadiazine among the crews of a number of U. S. naval ships in San Pedro Bay, P. I., during an outbreak of *Shig. flexneri 3* dysentery. The results were inconclusive as other control measures were instituted at the same time. In a dysentery outbreak in a U. S. Navy cruiser, Mount and Floyd (1948) gave sulfadiazine to all members of the crew on the tenth day of the outbreak. Prompt subsidence of the epidemic was not considered conclusively due to prophylaxis. In an epidemic of Sonne dysentery among a group of children in an institution, sulfaguanidine was found to be effective prophylactically

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when the results were compared with untreated control groups (Yannet *et al.*, 1944).

No reports have been seen of trials of antibiotics as prophylactic agents against shigellosis.

It is apparent, from the results cited above, that the question of the effectiveness of drug prophylaxis against shigellosis has not been answered, inasmuch as a controlled experiment with constant challenge has not been reported. The Conference on Bacillary and Amebic Dysentery, Division of Biological Sciences, National Research Council (1952) noted that most arguments in favor of prophylaxis against shigellosis were based on extrapolation from therapeutic studies, and recommended that a controlled study be done in an endemic area. The present report is concerned with such a study.

MATERIALS AND METHODS

The experiment was designed to determine the prophylactic effect of sulfadiazine, dihydrostreptomycin and oxytetracycline on the *Shigella* infection rate and the incidence of diarrheal disease in a group of children receiving the agents, as compared with a control group of subjects. As described previously, 300 children had been selected for study in a village near Cairo. All were between one month and five years of age and lived under similar environmental conditions. One hundred were placed at random in a group to receive prophylactic agents. These children, in turn, were randomly divided into a "Sulfadiazine Group" (33 children), a "Dihydrostreptomycin Group" (34 children) and a "Oxytetracycline Group" (33 children). A second hundred subjects were designated as the "Control Group". Age and sex data of the control and prophylaxis groups are shown in Table 1.

The experiment was started during the first week of April and continued until the end of October, which period had been previously observed to be the season of highest endemicity of, and therefore of greatest natural challenge by, *Shigella* in this environment.

During this 32-week period of observation the children in both control and prophylaxis groups were visited weekly by a nurse or technician. During the visit the rectal temperature was recorded, the mother questioned as to the number of stools during the previous 24 hours, the general condition of the child

TABLE 1

Age and sex data of children in control and prophylaxis groups

Group	Number	Male	Female	Mean Age Months
Control	100	51	49	27.8
Sulfadiazine	33	20	13	25.3
Dihydrostreptomycin	34	14	20	28.7
Oxytetracycline	33	22	11	26.2

TABLE 2

Dosage of prophylaxis agents. 3 weekly doses

Drug	Up to 1 yr. of age	Up to 2 yrs. of age	Up to 3 yrs. and over
Sulfadiazine	0.3 gm.	0.6 gm.	0.9 gm.
Dihydrostreptomycin sulfate (Pfizer)	0.15gm.	0.3 gm.	0.45gm.
Oxytetracycline (Terramycin, Pfizer)	0.3 gm.	0.6 gm.	0.9 gm.

noted and a rectal swab culture taken. Children in both control and prophylaxis groups received oral vitamin therapy once weekly, consisting of 5,000 U.S.P. units of vitamin A, 400 U.S.P. units of vitamin D, 75 mg. ascorbic acid, 2 mg. thiamine hydrochloride, 3 mg. riboflavin and 20 mg. nicotinamide. The test prophylactic agents were given orally to the prophylaxis groups during this visit and twice again on alternate days in the same week. The dosages of the sulfonamide and antibiotics are listed in Table 2. During the latter two visits to administer the prophylactic agents, the nurses or technicians did not make clinical observations or rectal swab cultures unless the children were ill.

Children in all groups who were ill were referred to the physician and were visited daily, as far as possible, by the nurse or technician. In both the control and prophylaxis groups severe diarrheal disease was treated with bismuth mixture, sulfa drugs, dihydrostreptomycin, oxytetracycline and chlortetracycline. Children in the prophylaxis groups, when treated for diarrheal disease, were given a drug other than the agent being tested in that individual.

At the laboratory, isolation and identification of *Shigella* organisms from the rectal swab cultures was done by the methods described in Section I.

It was anticipated that the prolonged use of the prophylactic agents in this study might lead to the development of strains of *Shigella* which were resistant to the sulfonamides and antibiotics. To determine the presence of such strains a majority of the cultures of these organisms isolated from the children on prophylaxis were tested for sensitivity, by the disc method, to the drug being used in each case. With cultures from the sulfadiazine group, filter paper discs saturated with an aqueous solution containing 100 mcg. of the drug per ml. were used. Bacto sensitivity discs (Difco) containing 100 mcg. of dihydrostreptomycin and 60 mcg. of oxytetracycline were used with cultures isolated from the other two groups. Sensitivity was defined as 5 mm. or more of growth inhibition around the discs. The results were compared with similar data from *Shigella* cultures recovered from the control group.

The prophylactic agents under test in this study had been reported to produce changes in the normal intestinal flora of humans (Baker and Pulaski, 1950; Bierman and Jawetz, 1951; Dalton, 1948; Dearing and Heilman, 1950; Dearing and Needham, 1953; Janbon *et al.*, 1952; Rivera and Sborov, 1951; Spaulding *et al.*, 1949). Since these reports were usually concerned with short

courses of the drugs, given in therapeutic doses, it was felt that it would be of interest to determine some of the effects on the intestinal flora of long term prophylaxis with sulfadiazine, dihydrostreptomycin and oxytetracycline. It was not possible to make exact qualitative and quantitative studies on the intestinal flora of the children during the entire period of the study. Observations were restricted to the incidence and approximate densities of those organisms that would grow on the selective media used for isolation of *Shigella*. In addition, for two weeks prior to the discontinuation of prophylaxis, and for three weeks afterward, the aerobic intestinal flora of the children in each of the prophylaxis group and a similar number from the control group was studied qualitatively by blood agar plate cultures.

RESULTS

During the 32-week period of study, the number of subjects had diminished, by attrition, to 88 in the control group, 27 in the sulfadiazine group, 29 in the dihydrostreptomycin group and 31 in the oxytetracycline group. Seven (7 per cent) of the children died in the control group. Five of the deaths were due to diarrheal disease of unknown etiology, one was attributed to diarrheal disease of unknown etiology and measles, and one was due to pneumonia. In the sulfadiazine group two (6 per cent) of the children died. Both of these deaths were associated with diarrhea of unknown etiology. In the dihydrostreptomycin group there were two deaths (6 per cent). One was attributed to diarrheal disease of unknown etiology and the other to pneumonia. There were no deaths among the children in the oxytetracycline group. There had been isolations of *Shigella* organisms in weeks previous to the deaths of some of the children who died from diarrheal disease, but it was not possible to relate these infections causally to the deaths. No toxic or other side reactions to any of the prophylactic agents was noted during the course of the study.

The collection of rectal swab cultures by nurse-technicians was satisfactory. Of the specimens scheduled to be delivered to the laboratory, 97.3 per cent were collected from the control group, 95.1 per cent from the sulfadiazine group, 92.1 per cent from the dihydrostreptomycin group and 89.5 per cent from the oxytetracycline group.

Effect of prophylaxis on total incidence of diarrheal disease. The total incidence

TABLE 3

Total diarrheal episodes in prophylaxis and control groups

Group	Number Subjects	Number Episodes	Av. Number Per Subject
Control	88	184	2.0
Sulfadiazine	27	48	1.7
Dihydrostreptomycin	29	41	1.4
Oxytetracycline	31	52	1.7

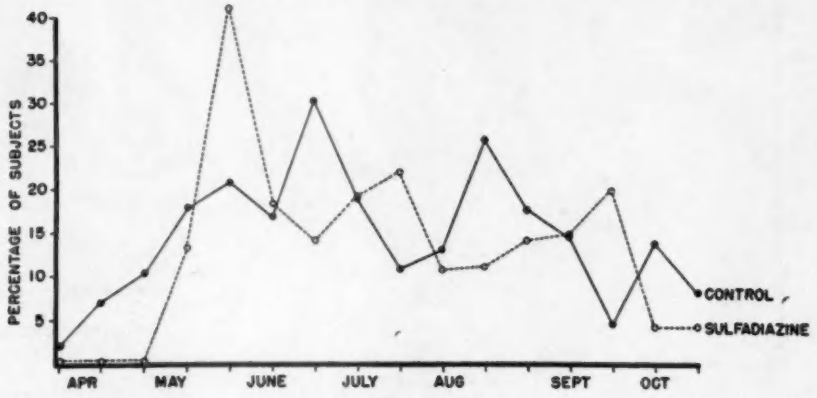

FIG. 1. Total incidence of diarrheal disease. Sulfadiazine vs. control group by bi-monthly periods.

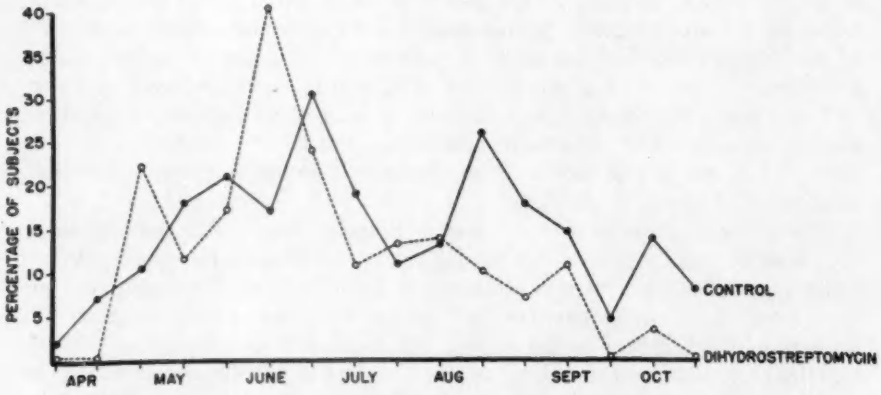

FIG. 2. Total incidence of diarrheal disease. Dihydrostreptomycin vs. control group by bi-monthly periods.

of diarrheal episodes in the prophylaxis groups, as compared to the control group, is shown in Table 3. The average number of episodes in the sulfadiazine group (1.7 per child) and in the oxytetracycline group (1.7 per child) is not significantly different from that of the control group (2.0 per child). There were fewer average episodes (1.4 per child) in the dihydrostreptomycin group.

The incidence, by bimonthly periods, of diarrheal episodes in the control and prophylaxis groups is shown in Figures 1, 2 and 3. No significant divergence of the curves, indicating marked effect of the prophylactic agent on the incidence of diarrheal episodes, is noted.

Effect of prophylaxis on incidence of diarrheal disease associated with Shigella. The average numbers of diarrheal episodes associated with *Shigella* that occurred

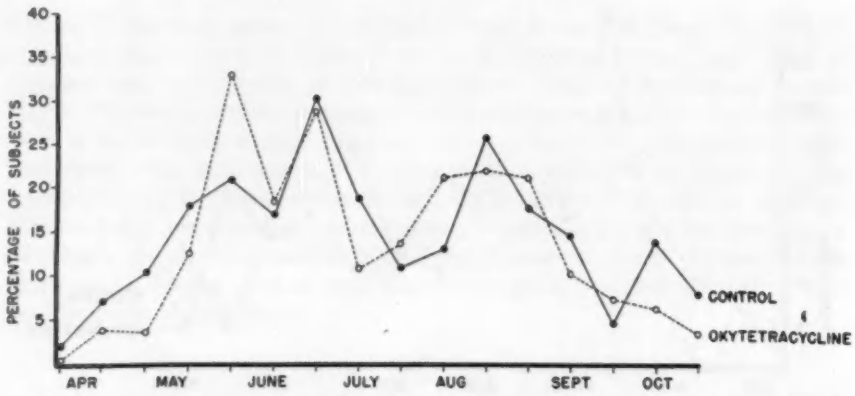

FIG. 3. Total incidence of diarrheal disease. Oxytetracycline vs. control group by bimonthly periods.

in the control and prophylaxis groups are shown in Table 4. The average number in the sulfadiazine group (0.9 per child) is not appreciably different from that of the control group (0.8 per child). The decreased incidence of the episodes in the dihydrostreptomycin group (0.4 per child) and in the oxytetracycline group (0.4 per child) is twofold. The incidence, by bimonthly periods, of diarrheal disease associated with *Shigella* in prophylaxis and control groups is shown in Figures 4, 5 and 6. The curves of the prophylaxis groups generally follow that of the control group.

Effect of prophylaxis on total incidence of Shigella *infections.* A comparison of the effect of prophylaxis on the total incidence of *Shigella* infections without respect to clinical symptoms is presented in Table 5. It can be seen that there were more *Shigella* infections per child in the sulfadiazine group than in the other prophylaxis groups or the control. This increase in incidence is not significant by chi-square analysis ($p = > .05$). The data of the dihydrostreptomycin group resemble those of the control group. There was, however, a marked reduction in the average number of *Shigella* infections per child in the oxytetracycline group. This difference, analyzed by chi-square, has statistical significance ($p = < .01$).

TABLE 4

Diarrheal episodes associated with Shigella in prophylaxis and control groups

Group	Number Subjects	Number Episodes	Av. Number Per Subject
Control	88	74	0.8
Sulfadiazine	27	26	0.9
Dihydrostreptomycin	29	14	0.4
Oxytetracycline	31	14	0.4

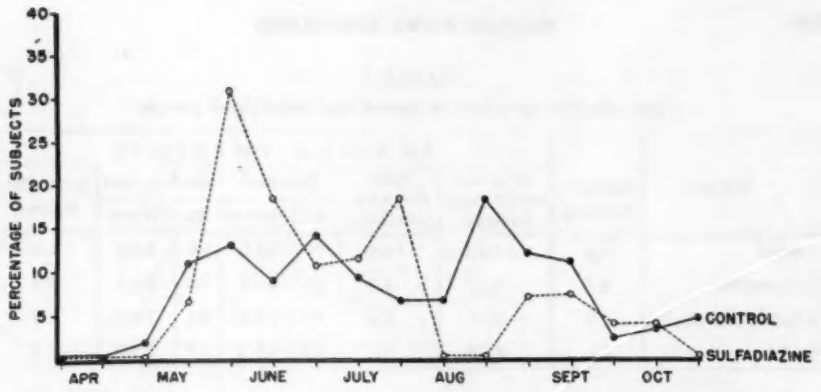

FIG. 4. Incidence of diarrheal disease associated with *Shigella*. Sulfadiazine vs. control group by bi-monthly periods.

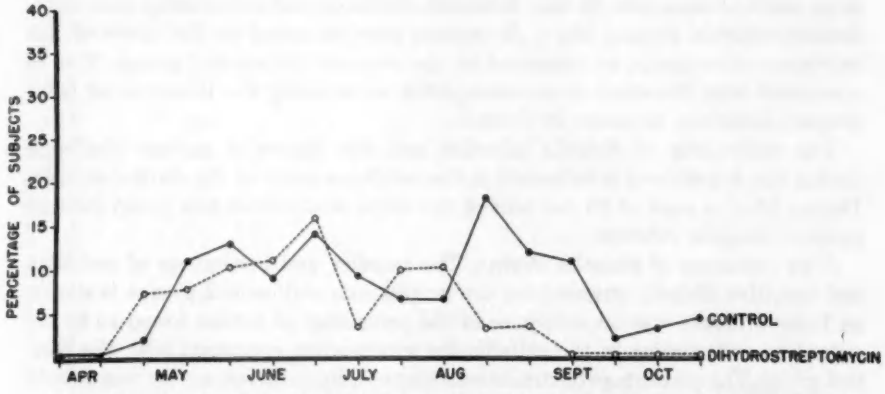

FIG. 5. Incidence of diarrheal disease associated with *Shigella*. Dihydrostreptomycin vs. control group by bi-monthly periods.

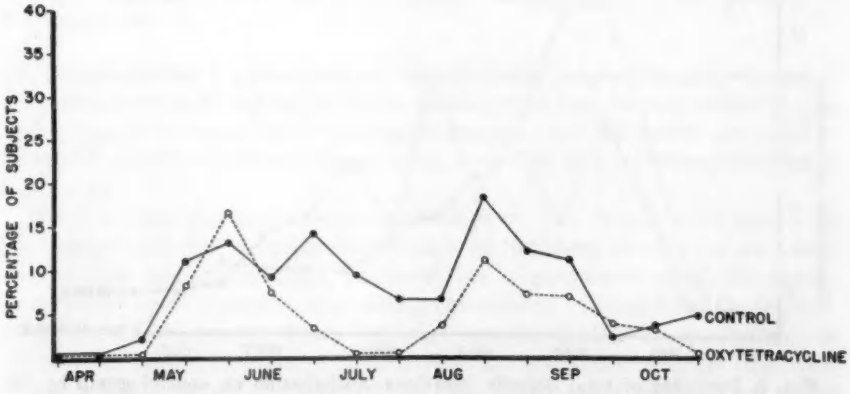

FIG. 6. Incidence of diarrheal disease associated with *Shigella*. Oxytetracycline vs. control group by bi-monthly periods.

TABLE 5

Total Shigella infections in control and prophylaxis groups

Group	Number Subjects	SHIGELLA INFECTIONS						
		Total No. Rectal Swab Cultures	Total Shigella Infections	Diarrheal		Non-Diarrheal		Av. No. Per Subject
				No.	Percent	No.	Percent	
Control	88	2740	168	74	44.0	94	56.0	1.9
Sulfadiazine	27	822	62	26	41.9	36	58.1	2.2
Dihydrostreptomycin	29	855	50	14	28.0	36	72.0	1.7
Oxytetracycline	31	888	31	14	45.1	17	54.9	1.0

The incidence of total *Shigella* infection in the prophylaxis groups, as compared to the control group, by bimonthly periods, is shown in Figures 7, 8 and 9. There is no marked deviation in the incidence curves of the sulfadiazine and dihydrostreptomycin groups, but a divergence may be noted in the curve of the oxytetracycline group, as compared to the curve of the control group. This is consonant with the effect of oxytetracycline in reducing the incidence of total *Shigella* isolations, as shown in Table 5.

The endemicity of *Shigella* infection and the degree of natural challenge during this experiment is indicated in the incidence curve of the control sample. During May, a peak of 25 per cent of the rectal swabs from this group yielded positive *Shigella* cultures.

Drug resistance of Shigella *strains.* The number and percentage of resistant and sensitive *Shigella* strains from the prophylaxis and control groups is shown in Table 6. There was no difference in the percentage of strains found to be resistant to sulfadiazine in the sulfadiazine group when compared with the control group. The percentage of strains resistant to dihydrostreptomycin was double that found among *Shigella* isolated from the control group. There was more than

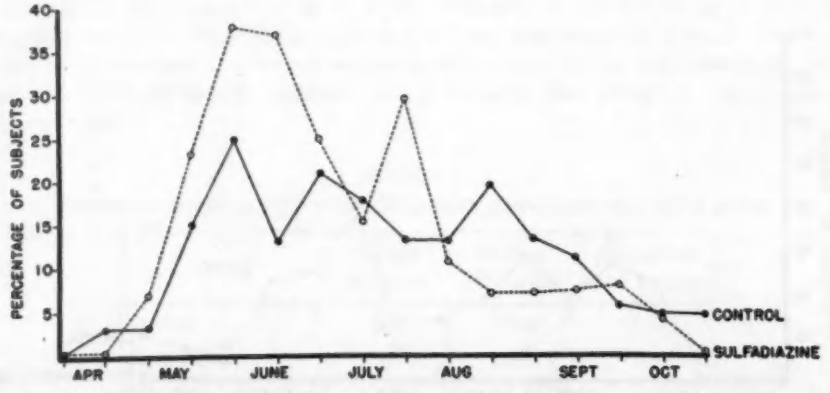

FIG. 7. Incidence of total *Shigella* infections. Sulfadiazine vs. control group by bimonthly periods.

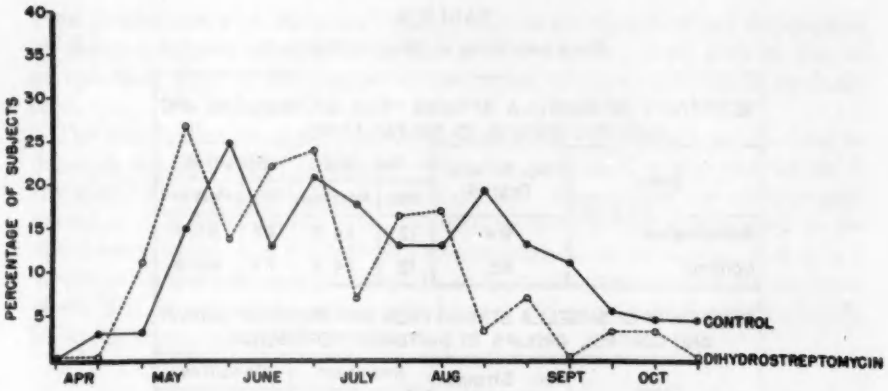

FIG. 8. Incidence of total *Shigella* infections. Dihydrostreptomycin vs. control group by bi-monthly periods.

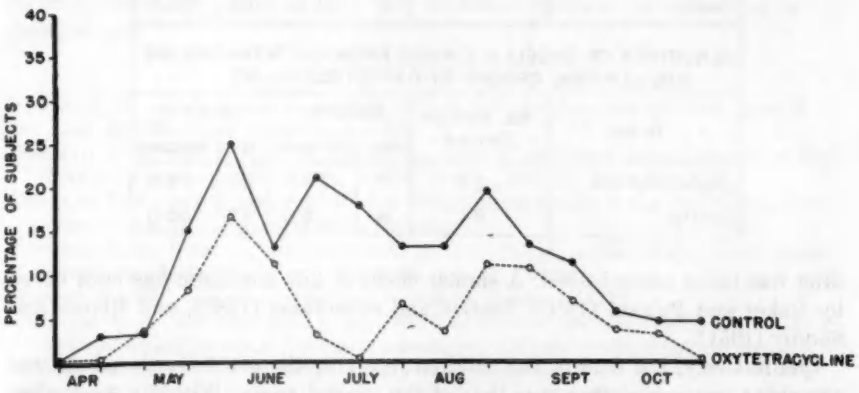

FIG. 9. Incidence of total *Shigella* infections. Oxytetracycline vs. control group by bi-monthly periods.

a four-fold difference in percentage of *Shigella* strains resistant to oxytetracycline from the group receiving the antibiotic as compared with *Shigella* from the control group. When examined by chi-square analysis these differences are found to be significant (dihydrostreptomycin group, $p = < .05$ and oxytetracycline group, $p = < .01$).

Effect of prophylaxis on normal intestinal flora. The prophylactic agents, in the dosage used, did not noticeably reduce the numbers of coliform organisms at any time during the study. The incidence of paracolon bacilli, *Alkaligenes* and *Pseudomonas* organisms was usually the same in cultures from the prophylaxis and control groups. *Proteus* species were isolated about three times as often from children receiving oxytetracycline as from subjects in the other prophylaxis groups or the controls. This predominance lasted only as long as the

TABLE 6

Drug sensitivity of Shigella strains

SENSITIVITY OF SHIGELLA STRAINS FROM SULFADIAZINE AND CONTROL GROUPS TO SULFADIAZINE					
Group	No. Strains Tested	Resistant		Sensitive	
		No.	Percent	No.	Percent
Sulfadiazine	84	12	14.2	72	85.8
Control	85	12	14.2	73	85.8
SENSITIVITY OF SHIGELLA STRAINS FROM DIHYDROSTREPTOMYCIN AND CONTROL GROUPS TO DIHYDROSTREPTOMYCIN					
Group	No. Strains Tested	Resistant		Sensitive	
		No.	Percent	No.	Percent
Dihydrostreptomycin	61	6	9.8	55	90.2
Control	109	5	4.6	104	95.4
SENSITIVITY OF SHIGELLA STRAINS FROM OXYTETRACYCLINE AND CONTROL GROUPS TO OXYTETRACYCLINE					
Group	No. Strains Tested	Resistant		Sensitive	
		No.	Percent	No.	Percent
Oxytetracycline	29	7	24.1	22	75.9
Control	81	4	5.0	77	95.0

drug was being administered. A similar effect of this antibiotic has been noted by Baker and Pulaski (1950), Janbon and co-workers (1952), and Rivera and Sborov (1951).

Qualitatively, the flora of the children receiving the prophylactic agents was somewhat less varied than was that of the control group. Within a week after discontinuation of the drugs this difference disappeared.

DISCUSSION

From the data presented here it must be inferred that the test agents, sulfadiazine, dihydrostreptomycin and oxytetracycline, in the dosage schedules given, were not effective or practical agents for the prevention of diarrheal disease associated with *Shigella*, or of *Shigella* infections, under the conditions of the experiment. Certain differences were noted between the data obtained from the prophylaxis groups and that from the control group, some of which were statistically significant. It is clear, however, that an effective and practical prophylactic agent must produce clear cut and positive results and not merely a statistically significant effect. Such an agent should prevent or suppress virtually all disease associated with *Shigella*, and this result was not obtained with any of the agents tested.

It must be emphasized that the observations of this study apply only to long

term prophylaxis with the stated dosage, and do not furnish direct information on the prophylactic effect of these agents over a short period, such as that of an epidemic. Further information on this aspect of the subject would be desirable.

The small numbers of subjects in the respective prophylaxis groups tend to diminish the meaning of comparison with the controls. However, the length of the study resulted in weekly, or more frequent, observations over an aggregate period of 13.5 person-years in the sulfadiazine group, 14.5 person-years in the dihydrostreptomycin group and 15.5 person-years in the oxytetracycline group. These periods would appear adequate to have detected marked prophylactic effect, if it existed, of the agents tested under the natural challenge by *Shigella* to which these children were exposed.

SUMMARY

Sulfadiazine, dihydrostreptomycin and oxytetracycline were tested as prophylactic agents over a period of 32 weeks in children living in an environment of high endemicity of shigellosis, and were not effective and practical in the dosages used.

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ATYPICAL CHRONIC INTESTINAL SHIGELLOSIS

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It is well known that chronic intestinal amebiasis commonly occurs in forms in which dysentery and diarrhea are altogether absent, and that in fact these may well represent the more typical forms of the disease, characterized by abdominal pains of variable intensity and localization, constipation, flatulence and dyspepsia rather than by diarrhea or dysentery. The occurrence of this atypical symptomatology in chronic intestinal shigellosis is, however, insufficiently recognized by the profession. During his routine clinical work, one of us came across a case of chronic constipation, left abdominal pain and flatulence in which repeated ordinary stool examinations failed to demonstrate any of the intestinal pathogens, while a stool culture was positive for *Shigella flexneri*. With specific treatment, the stool culture became negative and at the same time the symptoms cleared. The patient gave no history of dysentery or diarrhea either preceding or accompanying the course of his illness.

Looking through the available mass of literature, we could find only scattered references to these atypical forms of chronic intestinal shigellosis. D'Antoni in his paper, "Diagnostic and therapeutic considerations in chronic shigellosis," has pointed out that "some patients with chronic shigellosis suffer from constipation and some may have normal bowel habits, and neither diarrhea nor dysentery is necessarily present. . . ." Silverman and Friedrichs (1943) have cited two cases of chronic intestinal shigellosis in one of which severe abdominal pain, and in the other obstinate constipation, was the only symptom.

A study of shigellosis seemed indicated to determine the relative frequency of cases with these atypical symptoms as compared with those having chronic diarrhea or dysentery among patients coming under our observation during a given period, and to note the clinical and therapeutic aspects of the condition.

METHOD

Intestinal shigellosis may be diagnosed either directly by identifying the organism in stool cultures, or indirectly by demonstrating specific agglutinins in the patient's serum or the specific bacteriophage in his stools.

It is recognized, however, that the agglutinin reaction has uncertain diagnostic value (Mackie and his associates, 1938; Manson-Bahr, 1939; Strong, 1945; and many others) and it is likely that it might be even less helpful in the atypical forms in which the symptoms indicate a less invasive infection. As for the phage method, this would be profitable only in recent cases, and furthermore it appears to lack specificity (Feemster, 1934; Wheeler and Burgdroff, 1941; and others). Since the value of the present study depended on an unequivocal diagnosis, it was decided to use only direct cultures.

TECHNIQUE

About 5 gm. of recently passed stools, including portions containing mucus or blood if any, were thoroughly emulsified in about 25 cc. of sterile saline in a conical flask and left for about 15 minutes for the heavy particles to settle. The supernatant fluid was then centrifugalized at high speed for about 15 minutes and the sediment used for cultivation.

Highly selective media which inhibit the concomitant intestinal organisms have to a certain extent an inhibitory effect on *Shigella* as well. For this reason, we preferred the less selective MacConkey bile salt lactose agar medium in the form of plates. Two plates were used for each case. Five standard loopfuls of the prepared stool were streaked on the surface of the first plate, and the same loop was used to streak the second with the residual material. Both plates were then incubated aerobically at 37°C.

Cultures were examined after 24 hours, but suspicious colonies were not picked off until after 48 hours' incubation, since it was noted that some genuine lactose fermenters (*Bacillus aerogenes* in particular) did not develop their typical red color except after 48 hours. The isolation and identification of the organisms were carried out in the usual manner including serological diagnosis by stock specific antisera.

RESULTS AND DISCUSSION

The stools of 426 cases with atypical symptoms and of 392 cases with diarrhea and dysentery were examined for *Shigella*. For certain reasons, only one stool sample was cultivated from each case. The results are shown in Table 1.

For clinical purposes, cases with atypical symptoms were divided into groups according to their symptoms and the incidence of shigellosis was estimated in each group separately. For the sake of comparison, the incidence of amebiasis in similar groups of cases studied by the writers is given in the same table. For the detection of *Entamoeba histolytica* in these cases the zinc sulfate flotation method (Faust *et al.*, 1938) was used and at least four stool samples from each case were examined.

From the table it can be seen that during the period of observation, 32 cases of atypical chronic intestinal shigellosis were diagnosed as compared with 16 *Shigella* infections with chronic dysentery and 4 with chronic diarrhea. The study was carried out on cases seen in private practice in which the incidence of dysentery is probably lower than in the general population, so that the figures may not reflect the true proportion of "typical" to "atypical" cases, but the results are informative nevertheless. The fact that the percentage of positives in the total dysentery group of cases (15%) exceeds that in the atypical group (8%) might be due to the greater frequency with which positive results can be obtained from dysenteric as compared with nondysenteric stools, rather than to a higher incidence of shigellosis in the dysenteric group (Topley and Wilson, 1936; Blacklock and Guthrie, 1937).

Among the cases of chronic intestinal amebiasis, the number of atypical cases diagnosed over a certain period exceeded those with dysentery or diarrhea. In

TABLE 1

Comparison of incidence of Shigella *and of amebic infections in cases with chronic intestinal symptoms which did and which did not have diarrhea or dysentery*

Symptoms	Number of Cases Whose Stools Were Cultured	Number of Positive Cases	%	Number of Cases Whose Stools Were Examined for Ameba	Number of Positive Cases	%
A. Cases without chronic diarrhea or dysentery						
Chronic colonic pain	52	8—Flexner	16	190	101	53
Combination of pain and flatulence	220	14 (10—Flexner; 4—Sonne)	6	334	172	51
Chronic constipation	24			54	33	61
Flatulence	26	2—Flexner	8	46	17	37
Combination of pain and flatulence and constipation	56	2—Flexner	4	54	29	54
Combination of pain and constipation	12	2—Dispar	17	21	10	50
Combination of constipation and flatulence	20			22	12	54
Dyspepsia	16	4 (2—Flexner; 2—Sonne)	25	75	37	50
Total	426	32	8	796	411	52
B. Cases with chronic diarrhea or dysentery						
Chronic dysentery	108	16 (10—Flexner, 4—Sonne; 2—Dispar)	15	83	33	39
Chronic diarrhea	284	4 (2—Flexner; 2—Sonne)	1.5	153	59	37
Total	392	20	5	236	92	38

the amebic group the percentage of positive cases in both the atypical and diarrhea-dysentery groups is higher than that in the *Shigella* groups. This is certainly due, at least in part, to the more efficient method of examination adopted for the former in which not less than four samples of stools from each case were examined by the zinc sulfate method, while only one stool sample from each case was cultured for *Shigella*. For the proper estimation of the actual incidence of shigellosis among these groups of cases, repeated stool cultures as well as additional methods including agglutination and phage methods have necessarily to be employed.

Type of organism in atypical shigellosis. Of the 32 positive cultures in the present series, 24 were of the Flexner, 6 of the Sonne, and 2 of the Dispar types. Although epidemics of acute bacillary dysentery due to the Shiga bacillus have

been reported in Egypt (Khouri 1902; 1927), no Shiga organisms were detected in the present cases. Various reports indicate that Shiga carriers are rare (Shiga, 1906; Fletcher, 1918; Saisawa and Tanabe, 1926). The types of organisms thus appear to be similar both in the carrier and the atypical states.

Association with other intestinal pathogens. A. *Entamoeba histolytica.* Six of the *Shigella*-positive stools also contained cysts of *E. histolytica.* The clinical picture was apparently not modified by the presence of the ameba, although in such cases it is difficult to assess the role of one pathogen in the presence of the other, since either or both might be active. In atypical shigellosis, unlike cases of chronic *Shigella* dysentery, sigmoidoscopic and serologic findings are not helpful in establishing the diagnosis. Probably failure to recognize concomitant *Shigella* infections in intestinal amebiasis explains the failure of thorough antiamebic therapy in some cases.

B. *Giardia lambia.* Cysts of *Giardia* were present in one case which also harbored *E. histolytica.*

C. *Helminths. Heterophyes heterophyes* eggs were present in one case.

Character of the stools in atypical shigellosis. To the naked eye they were normal except for hardness in the constipated cases. On microscopic examination, apart from a variable increase in food debris, there were no abnormal findings and particularly no cellular exudates.

CLINICAL OBSERVATIONS

In the present group of 32 cases there were 26 males and 6 females between 20 and 45 years of age. A history suggestive of an antecedent acute bacillary dysentery was obtained only in 4 cases, while in the others there was no such history and the onset of symptoms was insidious. The initial attack, however, might have been so mild that it was overlooked. The duration of the condition in the present group was 2–3 years, though it was difficult in some cases to fix the date of onset.

The course was usually protracted and typically irregular, periods of activity alternating with periods of relative or even complete quiescence. The abdominal symptoms include pain, flatulence, constipation and dyspepsia, in any combination. The cases are grouped according to the dominant symptoms in Table 1, but it must be emphasized that most of the symptoms recurred to some extent in all.

Pain was more commonly dull with occasional paroxysmal exacerbations rather than colicky, and was referred to both sides as often as to the left side only. *Flatulence* was present in varying degree in almost all the cases, especially after meals. Gurgling on palpation occurred as frequently as in amebiasis, so that Manson's sign, "the amebic splash," is not specific only to amebiasis. *Constipation* varied in severity and might be interrupted by attacks of diarrhea and even frank dysentery. *Dyspepsia* occurred in a majority of the cases, but nausea and vomiting were rare and if marked were usually due to associated neurosis. Epigastric pain (probably of reflex origin) was sometimes so severe that peptic ulcer or gall bladder disease were simulated. *Palpation* of the abdo-

men elicited no localization of tenderness or spasticity which might not also have accompanied amebiasis (Manson-Bahr, 1939) or bacillary dysentery. Chronic atypical intestinal shigellosis has therefore no characteristic clinical picture. Mistaken diagnoses of both amebiasis and irritable colon are undoubtedly being made, and differentiation rests only on stool examination.

Sigmoidoscopic findings. Sigmoidoscopy was done in 18 cases. In the majority, the appearance of the mucosa was normal. In some cases variable degrees of hyperemia were seen and in some other cases atrophy of the mucosa with ready visualization of the blood vessels was observed. A few small and superficial ulcerations were seen only in two cases.

Roentgenographic findings. Roentgenography was done in 10 cases, in all of which it showed partial loss of haustration and marked spasticity of the colon.

General features. These include fatigue, lassitude, physical and mental asthenia, dizziness, headache, lack of concentration, insomnia, irritability, palpitation, backache, general body ache and vasomotor disturbance. The weight and the general condition remained, however, generally good. These symptoms are essentially those grouped under "neurasthenia" and are in no way typical for shigellosis nor, as Craig (1944) pointed out, for amebiasis. They were present in a minor degree in almost all the cases but were specially marked in only one-third of them. In D'Antoni's experience, this syndrome is presented by 75–90 per cent of all patients with chronic shigellosis. The relations between such general features and chronic intestinal shigellosis is as problematic as in intestinal amebiasis. It is known that nervous impulses playing on the colon can produce abdominal symptoms and signs quite similar to those of chronic intestinal shigellosis and amebiasis—the irritable colon.

The association of such general disturbances with a stool which is positive for *Shigella* or *E. histolytica* could be due to 1) coincidence, i.e., a neurasthenic patient contracting an intestinal infection, or a case of shigellosis or amebiasis developing an unrelated neurasthenia; 2) abdominal symptoms in a neurasthenic individual who happened to be at the same time a carrier of *Shigella* or amebae; or 3) neurasthenia developing as a result of the infection. The problem is a difficult one for the physician and has obvious therapeutic implications. Evidence of activity of the intestinal condition would favor the third possibility, while complete absence of activity favors the second, although the existence of the carrier state is less certain in shigellosis than in amebiasis. It is now known that the sedimentation rate will rise if the intestinal condition is active, even in the absence of other signs; unfortunately it was not determined in the present series.

Although on theoretical grounds, a toxic mechanism may be operating from the intestine, we believe from clinical experience that neither intestinal shigellosis nor amebiasis can initiate the neurasthenic syndrome; they simply manifest a latent neurasthenia. This would explain why therapy of the intestinal condition is inconstant in its effect on the neurasthenic symptoms.

The liver in atypical chronic intestinal shigellosis. In many of our cases the liver was slightly or moderately enlarged and its consistency was firm. The intimate anatomic relation between the liver and intestines makes it quite possible for

the former to be involved in shigellosis. The liver might be damaged by excretion of the bacilli, or affected by toxins as in chronic intestinal amebiasis. The nature and degree of the damage have not been assessed, but it is possible that some of the neurasthenic symptoms are due to hepatic involvement.

True incidence of atypical shigellosis in the present series. Since in the present study only one stool sample was cultured from each case, the number of positive stool cultures must be taken to represent only a very small minority of the true number of cases of intestinal shigellosis for the following reasons:

1. The incidence of positive fecal cultures in acute bacillary dysentery regularly falls to a low level a few days after onset, and, a few weeks later it becomes extremely difficult and sometimes impossible to isolate the organism from fecal cultures. It can easily be conceived how much more difficult such isolation becomes in chronic bacillary dysentery. Manson-Bahr (1939) succeeded in isolating dysentery bacilli from the feces in only three out of 107 known cases of chronic bacillary dysentery at the Hospital for Tropical Diseases, London. This difficulty is most marked when we are dealing with non-dysenteric non-diarrheic stools (Blacklock and Guthrie, 1937; Topley and Wilson, 1936).

2. It is well known that the excretion of dysentery bacilli in chronic cases or carriers may be intermittent and the bacilli may remain absent in the stools for many months (Bockus, 1947).

3. Isolated stool examinations are of very little value and repeated cultures on many successive days are essential (Hiss, 1916; Silverman and Friedrichs, 1943; and others). All agree that the dysentery organisms can often be recovered from the mucosa after fecal cultures have been repeatedly negative, especially in chronic dysentery and in convalescent carriers.

In view of these considerations and of our own clinical experience we believe that atypical chronic intestinal shigellosis is very common in this country. The importance of recognizing these atypical cases cannot be overemphasized because immediate therapy may prevent a subsequent frank dysentery with possible disastrous complications such as perforation; spread of infection to others can be blocked; and extra-intestinal complications avoided. Two cases of the present series may serve as examples: in one with gall-bladder symptoms, bile culture revealed an almost pure culture of *Shigella sonnei*, while the other with symptoms referable to the urinary tract was proved by culture of the urine to have a *Bacillus dispar* infection.

The entity of atypical chronic intestinal shigellosis and its relation to other forms of the disease. How far are we justified in assuming that atypical chronic intestinal shigellosis is a separate entity? If we exclude the 6 cases with *E. histolytica*, there remain 26 cases with intestinal symptoms in which no other pathogens could be demonstrated than the *Shigella* organisms. The disappearance in the cases of both the organisms and the clinical syndrome after specific therapy makes it reasonable to attribute the symptoms to the *Shigella* infection. While the evidence is admittedly mainly clinical, it is essentially the same as that presented in authentic cases of atypical intestinal amebiasis.

Furthermore, the intermediate relationship which these cases bear to the

dysenteric state on the one hand and the carrier state on the other marks it as a distinct form of the disease. The relatively mild character of the symptoms and the good general condition of the patient as well as the sigmoidoscopic findings distinguish atypical shigellosis as a low grade, mainly superficial noninvasive form of the infection. We have observed that in some cases the symptoms spontaneously disappear for a time and the patient becomes a temporary carrier, while in other cases, symptoms may develop in a carrier under the influence of certain factors (mechanical, chemical, thermal, or other infections), which lower the local resistance of the intestine and thus break the host-parasite balance.

Many cases of atypical shigellosis were, in all probability, reported as carriers by previous authors who classified cases with positive *Shigella* cultures either as patients or carriers according to the presence or absence of diarrheal-dysenteric symptoms only. If such carriers had been subjected to strict inquiry or careful observation for some time, many of them would undoubtedly have been found to suffer from such atypical symptoms as those mentioned above. The fact that many authors reported the presence of intestinal lesions or abnormal stools in the majority of their series of carriers is not without significance in this respect (Manson-Bahr, 1939; Saisawa and Tanabe, 1926; Felsen, 1945; Fairley and Boyd, 1943; and Bockus, 1947).

The similarity in type of organism in the atypical and the carrier states further shows the relation between the two conditions. A true healthy carrier state in intestinal shigellosis has, in fact, been regarded with doubt, or has been considered exceedingly rare (Hardy and Watt, 1944). It is quite possible that the carrier state is simply an arrested phase of the atypical form.

Furthermore, it has been observed that in some cases of chronic bacillary dysentery with positive stool cultures the dysenteric symptoms give place to such atypical symptoms as pain, constipation and flatulence for variable periods (many months) after which the dysenteric features recur and alternation of dysenteric and atypical symptoms continues until treatment is given.

It is thus clear that the atypical state of chronic intestinal shigellosis may be taken to represent an intermediate state between the diarrheal-dysenteric state of virulent infection and the healthy carrier state. Since the occurrence of atypical chronic intestinal shigellosis denotes a certain degree of adaptation between the parasite and the host, it may be expected to prevail in the tropics where repeated subclinical infections are common.

Why has atypical chronic intestinal shigellosis remained generally unrecognized? The great difficulty experienced in isolating *Shigella* organisms from the stools is undoubtedly the main factor which has prevented recognition of the condition for so long. The relatively easier detection of *E. histolytica* in the stools has led to the earlier recognition of atypical forms of chronic intestinal amebiasis. Many cases of so-called post-dysenteric constipation and dyspepsia, considered by previous writers as sequelae of acute bacillary dysentery, are probably cases of atypical chronic intestinal shigellosis in which the *Shigella* organisms could not be identified. A misconception regarding the diagnosis of *Shigella* carriers has also been responsible for the lack of recognition of the condition. Many cases

of chronic atypical shigellosis have been considered as carriers for the simple reason that they had no dysentery or diarrhea.

THERAPY

The treatment adopted in the first half of the cases consisted of streptomycin 1 gm. daily parenterally (0.5 gm. every 12 hours) together with sulfathalidin 1 gm. T.D.S., for 10 days, followed by 10 days rest, after which the course was repeated. Later Terramycin was used in the rest of the cases in doses of 0.5 gm. every 6 hours for 8 days.

The results in both groups were quite similar. The immediate effect on the clinical picture could be described as excellent in the great majority, and curative in a few cases. However, a clinical relapse, partial or complete, occurred in almost all the cases some variable time afterwards. One stool culture was made from every case some time after the therapy and it was negative in every case, but the inadequacy of this procedure has been discussed.

SUMMARY

1. The stools of 426 cases suffering from chronic abdominal pain, flatulence, constipation and/or dyspepsia on one hand, and those of 392 cases suffering from chronic dysentery and diarrhea on the other, were examined over the same period of time for *Shigella* organisms. Only one stool sample was cultured from each case. Thirty-two cases in the former group and twenty cases in the latter group were found to have *Shigella* organisms in their stools. This condition of chronic atypical intestinal shigellosis was studied in detail from the clinical point of view, together with radiologic and sigmoidoscopic examinations in some cases. It is considered to be a distinct form of the disease intermediate between the carrier state and that of frank diarrhea or dysentery.

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INCIDENCE OF TULAREMIA AND ROCKY MOUNTAIN SPOTTED FEVER AMONG COMMON TICKS OF ARKANSAS

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Tularemia, a specific infectious disease of mammals caused by *Pasteurella tularensis*, is known primarily as a disease of rabbits and various genera and species of the order Rodentia. The disease is transmitted to man chiefly by contact with these infected animals, their tissues, and their ectoparasites. Epidemiological investigations by Washburn and Tuohy (1949) in Arkansas revealed that most of the human cases reported during the months of April through September had histories of tick bites. The number of rabbit-contact cases in Arkansas was shown by these studies to reach a peak in December or January and to decline by March. Subsequently, Hopla and Downs (1953) reported the finding of *P. tularensis* in lone star ticks, *Amblyomma americanum* collected in Polk County, Arkansas, in 1950 and Calhoun (1954) reported six lots of naturally-infected lone star ticks collected in Arkansas during 1951 and 1952.

The objectives of this paper are to report (a) additional collections of tularemia-infected lone star ticks; (b) results of Rocky Mountain spotted fever tests; (c) the incidence and distribution of infected ticks by sex, stage, and season.

METHODS AND PROCEDURES

Collection methods. Ticks were collected from vegetation by drags, i.e., by pulling a flag-like flannel cloth, 1 yd. x 1½ yds., over the surface of shubbery or other vegetation likely to be infested for a distance of 100 paces, or approximately 100 yards, and by examination of small mammals, domestic and wild birds, dogs, cattle and horses from Garland, Hot Springs, Montgomery, Benton, Washington, Polk and Saline Counties (Table 1).

Small animals were placed in canvas bags immediately after capture and were left for several hours. The bags were then turned inside out and the animals and bags placed in a white enameled baby bath tub. The unattached ticks and other ectoparasites were removed and the animals combed and inspected for attached ectoparasites. After identification, ectoparasites were selected for laboratory tests and the living specimens were placed in plastic tubes. Specimens to be tested were shipped by air to a laboratory for animal inoculation tests. Tests were made by personnel of the San Francisco Field Station, Public Health Service, under guidance of Mr. Frank Prince and Dr. Stewart Quan. When feasible, pools of ticks were limited to a single stage, sex, locality, and host.

INCIDENCE OF TULAREMIA

Tularemia infection in the lone star tick. Eleven infected pools of lone star ticks were found during a period of one year (Table 2). The detectable percentage of infection was found to be quite low; in the 576 lone star tick pools composed of

TABLE 1

Number of lone star ticks and pools collected in Arkansas by host and tested for tularemia (April 14, 1952–May 1, 1953)

Host or Other Source	No. of Sources Examined	Total No. Pools Tested	Average Number Ticks per Pool	Number of Positive Pools
Aves				
Quail (*Colinus virginianus*)	6	4	48*	1
Drag	1,516	235	82	5
Mammalia				
Dog	234	168	25	2
House cat	1	1	1	0
Cow	79	56	37	1
Horse	13	12	27	0
Ground hog (*Marmota monax*)	1	1	32	0
Squirrels				
(*Sciurus niger*)	10	5	44	1
(*S. carolinensis*)	2	2	6	0
Harvest mice (*Reithrodontomys fulvenscens*)	1	1	1	0
Cottontail rabbit (*Sylvilagus floridanus*)	5	5	24	0
A number of host species	89	86	27	1†
Total	1,957	576	50	11 (1.9%)

* Read to nearest whole number.

† Positive pool number 3, table 2.

TABLE 2

Naturally tularemia-infected lone star ticks collected in Arkansas, April 1952–April 1953

Date Positive Pool Collected (1952)	Source of Collection Composing Pool	Location by County	Number of Specimens in Positive Pool by Stage or Sex				Pool‡ Number
			L.*	N.	F.	M.	
May 5	12 drags	Montgomery	—	—	15	—	1
May 26	2 dogs	Garland	—	—	38	—	2
July 11	2 cows,	Washington	—	1	27	36	3
	10 drags		—	1	—	—	
July 29	2 cows	Benton†	1	11	22	23	4
July 29	5 dogs	Benton†	12	11	7	31	5
Sept. 10	6 drags	Garland	200	—	—	—	6
Sept. 10	6 drags	Garland	—	95	—	—	7
Sept. 10	6 drags	Garland	500	8	—	—	8
Sept. 16	1 squirrel	Garland	49	4	—	—	9
Sept. 16	1 quail	Garland	65	4	—	—	10
Sept. 16	6 drags	Garland	50	38	—	—	11

* L. Larvae; N. Nymph; F. Adult Female; M. Adult Male.

† From same farmstead.

‡ Pools 1–5 reported in previous paper by Calhoun (1954).

28,661 individuals submitted for tests (Table 1), only 1.9 per cent of the pools were positive. A higher percentage (2.1 per cent) of infected pools occurred among ticks collected by drags than from any other source, probably due to the greater number of ticks per pool collected by this method.

Assuming there was only one infected individual to each positive pool, then one lone star tick of each 2,605 was naturally infected. If so, only about 0.04 per cent of the lone star tick population within the areas studied was naturally infected with *P. tularensis* to the extent detectable by the laboratory methods used. The large number of negative lone star tick pools from two Arkansas counties possessing high tick populations and human tularemia cases indicate the "spottiness" of the disease in nature.

Incidence of tularemia-infected ticks in known tularemia foci. The degree of spottiness in distribution of detectable tularemia is also indicated by findings in certain foci in Benton and Garland Counties, Arkansas. Subsequent to the finding of a human case and the positive collections (pools 4–5, Table 2) on a 40-acre farm in Benton County, intensive collections were made during August to determine the incidence of infection on that farm, which was divided into 14 small work units. Ticks were taken from each of these units. Collections were as follows: 6,645 lone star and 6 American dog ticks, *Dermacentor variabilis*, from 172 drags; 470 lone star and 218 American dog ticks from 15 dogs; 693 lone star and 1 American dog tick from cattle; and 158 lone star ticks from 2 squirrels. Most of the ticks collected by drags were in larval and nymphal stages. None of the 7,966 lone star or 225 American dog ticks collected from these sources was found to be tularemia-infected.

Again, two additional tularemia foci were located in Garland County, September 10, 1952, by "dragging", on a farm of 160 acres. Two positive pools (pools 6–7, Table 2) of lone star ticks were collected at one site and additional infected specimens were taken 6 days after the initial positive collections (Table 2, pool 11). A total of 1,434 lone star ticks and 6 American dog ticks collected by drags, and 9 rabbit ticks, *Haemaphysalis leporis-palustris*, from 2 cottontail rabbits were removed from this farm and tested for the presence of tularemia with negative results before collection of the two initial positive pools.

Although practically all of the central third of this 160-acre farm was cattle pasture and excellent tick habitat, the highest concentration of ticks was found in a small wooded area measuring approximately 75 square yards. Concentration of cattle within this small area for shade, as well as the high humid conditions along a wet-weather creek bed afforded excellent habitat for the large population of ticks found there. A seasonal decline in numbers of active ticks immediately followed the last positive collection, after which it was possible to collect only 439 more lone star ticks from the positive area for testing. None of the latter collections were found infected with tularemia.

A third tularemia focus on another 160-acre farm about 2 miles from the preceding farm was located by infected pool number 8 (Table 2). This farm was selected for extensive study because: (a) relatively few ticks had been collected before the finding of a positive pool, and (b) trapping records indicated that the

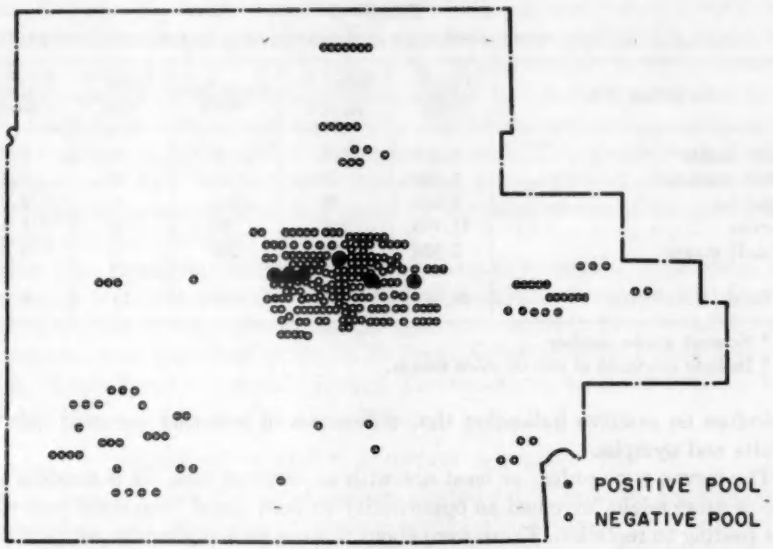

FIG. 1. Garland County, Arkansas, showing relative locations from which tularemia positive and negative pools of ticks were taken.

area was heavily populated by native mice and rats. No rabbits were observed or trapped in this study area. Only a small portion of this area was being used as a permanent pasture for cattle, but the dense vegetation and the high humidity maintained by a creek and its two branches flowing through the area provided a good habitat for tick populations. After the finding of positive pool number 8, the area which composed the tick habitat was divided into 4 work units for intensive collecting. Three hundred and forty lone star ticks and 26 American dog ticks were collected and tested before the seasonal decline in ticks hampered investigations. None was found to be tularemia-infected.

All told, 95 separate collections were made in Arkansas, with the exception of Garland County, in sites separated from each other by at least 1 mile, before one infected site was found during 1952 and 1953. Approximately 4,700 lone star ticks were taken. In Garland County, over 240 separate collections were made in 1952 in locating 7 positive sites (Figure 1).

Incidence of tularemia in the stages of the lone star tick. No adult male of the lone star tick was implicated separately as a carrier of tularemia in nature during these studies. Infected adult male specimens may, however, have been present in 3 of the 7 pools of mixed sexes which were found to contain an infected tick, or ticks (Table 3).

Over 2 per cent of the pools of adult females, 0.7 per cent of the pools of nymphs and 1.4 per cent of the pools of larvae were positive. However, the average number of nymphs per pool was twice that of the average number of adult females and about the same as that of adults of both sexes. There was

TABLE 3

Incidence of tularemia in sexes and stages of the lone star tick in Arkansas (1952–1953)

Sex or Stage Tested	Total No. of Ticks Tested	Average No. of Ticks per Pool	No. of Pools Tested	Number of Positive Pools	Per cent of Pools Found Infected
Adult males	1,754	19	93	0	0
Adult females	1,546	19	83	2	2.4
Nymphs	5,630	39	145	1	0.7
Larvae	11,775	168	70	1	1.4
Mixed† stages	7,956	46	165	7	4.2
Total	28,661	51	556	11	2.0

* Nearest whole number.

† Include mixtures of one or more stages.

therefore no positive indication that differences of infection occurred between adults and nymphs.

The larvae were unfed, at least not with an obvious meal. It is possible that one or more might have had an opportunity to draw blood from some host without feeding to repletion. There were about 9 times as many larvae per pool as of adult females (Table 3).

Parker and Spencer, 1926, have reported transovarian transmission of tularemia organisms with another species of tick, *Dermacentor andersoni*. Hopla, 1953, has shown that, when larvae become infected, they retain infection into the adult stage. There appears to be no previous indication that unfed larvae of the lone star tick may have been infected.

Seasonal incidence of tularemia infection in the lone star tick. Washburn and Tuohy (1949) have shown that human cases of tularemia with tick bite histories began to increase in March with a peak in May and an additional smaller peak in September. There is a sharp drop in the months of July and August.

During the investigations herein reported, the writers noted a seasonal drop in the activity of adult lone star ticks beginning in July and August, and an increase in the numbers or activity of larval and nymphal stages beginning in August and reaching a peak in September. Tularemia-infected lone star ticks were collected in May, July, and September, 1952, during the peak months of human tularemia cases. Positive ticks therefore were found during the periods of high adult activity and at the time of the smaller peak of human tularemia cases in September. However, relatively few ticks were taken during the winter, and it is quite possible that the absence of positive pools may have been due to small collections rather than to absence of tularemia in ticks.

Other ticks tested for tularemia. The American dog tick, although a suspect as a carrier of tularemia by association with case histories, was not found spontaneously infected until recent years. Green (1942) reports that less than 0.01 per cent of this tick in Minnesota were found to be naturally infected but that most of the cases are acquired by its bite. An insufficient number (2,627) was tested to determine whether or not this species is infected in Arkansas. There is, however, little reason to believe that it is not.

Rabbit ticks were removed and tested from both jack and cottontail rabbits, quail, and one wren during these studies. Although none was found infected, an insufficient number (2,897) was tested to be certain whether or not tularemia occurred, especially if detectable infection was as low as in the lone star tick.

The black-legged tick, *Ixodes scapularis*, is the most common tick attaching to man and animals in Arkansas during the winter months. This species has not been found spontaneously infected with tularemia. An insufficient number (846) black-legged ticks was collected and tested during these studies to determine the incidence of tularemia in it.

Other ticks tested for the presence of *P. tularensis* by animal inoculation, all with negative results, were the winter tick, *Dermacentor albipictus*, and *Ixodes baergi*, a tick found on swallows. Sample specimens of all ticks collected during these studies were identified by Dr. H. D. Pratt, Communicable Disease Center, Public Health Service, Atlanta, Georgia. The remainder were identified by the authors.

INCIDENCE OF ROCKY MOUNTAIN SPOTTED FEVER

In the lone star tick. Rocky Mountain spotted fever, induced by *Rickettsia rickettsii*, has been reported in increasing numbers in Arkansas for a number of years. Over a 13 year period (1940–1952), 54 have been reported in the state. The lone star tick has been shown to be an efficient vector of this disease under laboratory conditions by Parker *et al.* (1933). However, it was not until 1943 that Parker *et al.* (1943) reported the isolation of the spotted fever organism from a collection of lone star nymphs taken from vegetation in Oklahoma.

The spotted fever studies, here reported, were secondary to the tularemia investigations but the data indicate a higher detected incidence. Tests of animals taken in this study were made by members of the San Francisco Field Station. Ticks for testing were placed in a 37°C. incubator for 24 hours to activate the rickettsiae, removed from incubator, killed with cyano-A-gas, placed in mortars for macerating and then triturated. Five hundred units of penicillin per cubic centimeter of macerated tick material was added, and the suspension refrigerated overnight. On the following day, an equal volume of streptomycin solution was added and the suspension held at room temperature for one hour. From 1.0 to 2.0 cc. of the supernatant fluid was then injected intraperitoneally into the test animal.

Suspected positives (Table 4) were those in which guinea pigs showed temperatures of 102.8F. for two or more consecutive days and a complement fixation titer of 1:8 or higher, or a combination of these factors. Diagnoses were considered positive when temperatures, titer, clinical symptoms, pathology, and resistance occurred in response to challange inoculations with a virulent strain of Rocky Mountain spotted fever. A confirmed positive diagnosis is that based on the isolation of *R. rickettsi* from tissues of the test animal.

Lone star tick pools submitted for spotted fever tests revealed the possible presence of the causal organism in three instances. One suspected positive pool of lone star ticks was removed from a cottontail rabbit, and two definitely positive pools were collected by means of drags (Table 4). No stage or sex of this tick was

TABLE 4

Laboratory results of tick pools found with positive Rocky Mountain spotted fever reactions (Arkansas 1952)

County and Month Positive Pools Collected	Host or Source of Positive Pools	Tick Species and No. in Positive Pools								Highest Temp. Recorded	Highest Temp. after Challenge	Comp.‡ Fix. Titer	Results
		Amer. dog tick				Lone star tick							
		L*	N	M	F	L	N	M	F*				
										°F.	°F.		
Garland, June	Drags	—	—	2	6	—	—	—	—	103.5	—	1:16	Suspected positive
Washington, July	Drags	—	—	5	—	—	—	—	—	102.0	—	1:8	Suspected
Garland, July	Dogs	—	—	—	28	—	—	—	—	102.4	105.8	1:64	Confirmed positive
Garland, July	Cottontail rabbit	—	—	—	—	80	1	—	—	102.4	†	1:64	Suspected
Garland, July	Drags	—	—	5	6	—	—	—	—	103.0	†	1:32	Suspected
Washington, July	Drags	—	—	—	—	485	90	—	1	102.6	103.0	1:16	Positive
Garland, July	Drags	—	—	1	12	—	—	—	—	103.6	102.8	1:64	Positive
Benton, July	Drags	—	—	—	—	—	45	2	1	102.9	103.9	1:8	Positive

* L, Larva; N, Nymph; M, Adult Male; F, Adult Female.

† Killed in bleeding.

‡ The end point selected in this work for the CF titers was 2 or more. Nonspecific and anticomplementary controls were diagnosed either as negative or as insignificant reactions.

implicated separately as a possible carrier of spotted fever. Suspects or definitely positives were composed chiefly of nymphs and larvae. Of those pools tested, 3.8 per cent produced either suspected or definitely positive reactions in test animals.

In the American dog tick. The American dog tick has been known as a vector of spotted fever in eastern areas for a number of years. Among ticks in Arkansas, it is a seasonal pest of man second only to the lone star tick. Two of the dogs from which positive American dog ticks were removed in July also yielded tularemia-infected lone star ticks on the same day. Tests of four pools of this tick caused such reactions in the test animals as to indicate that they also were infected, but isolation of the spotted fever organism was not made in these instances.

In the rabbit tick. The rabbit tick was not implicated as a carrier of Rocky Mountain spotted fever by these studies but an insufficient number was tested to be conclusive.

SUMMARY

In the course of a year's field study in Arkansas, 576 pools totalling 28,661 lone star ticks (*Amblyomma americanum*) were tested for tularemia by animal inoculation, and 11 pools (1.9 per cent) were found positive—an average of one infected tick in 2,605, assuming only one infected individual to each positive pool. The distribution of naturally infected ticks was shown to be spotty, and the finding of a batch of infected, unfed larvae of *A. americanum* indicates the probable occurrence of transovarian passage of tularemia in this species.

The lone star tick was the only arthropod found naturally infected with tularemia, although the number of other ticks tested was insufficient to justify general conclusions. Tularemia-infected lone star ticks were collected in May, July, and September, coinciding with the reported seasonal incidence of human infections; and the data presented, taken in connection with previous epidemiologic investigations, point to ticks as the chief vectors of tularemia in Arkansas.

Pools of lone star, American dog, black-legged and rabbit ticks were tested by animal inoculation for Rocky Mountain spotted fever organisms (*R. rickettsi*), but only one successful isolation was made, from the tissues of a guinea pig inoculated from a pool of female American dog ticks. Reactions of several guinea pigs inoculated with material from pools of lone star ticks indicated rickettsial infections, but the organism was not recovered from the test animals.

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TUBE AND SLIDE TECHNIC IN THE HEMAGGLUTINATION OF *VIBRIO COMMA*

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Neter *et al.* (1952) observed that *E. coli* serotypes 055 and 0111 may be adsorbed on red blood cells. They devised a serial tube agglutination technic to demonstrate the presence of homologous antibodies in sera of patients infected with these organisms. Heat-treated *E. coli* cells adsorbed on mammalian and avian erythrocytes were used as antigens by these authors.

Agglutinins in cholera usually appear late and generally only in low titers. The possibility of utilizing the hemagglutination method for testing cholera sera was, therefore, worthy of investigation. Practical considerations suggested the comparison of the simpler slide technic with the tube method. Since sera from recent cholera cases were not available, it was necessary to set up trials with sera of rabbits vaccinated against different cholera vibrio types.

Three *V. comma* strains, belonging to the Inaba, Ogawa and Hikojima subgroups and rabbit sera prepared against them were used. Only "O" sera and antigens were employed, since all strains showed hemodigestive properties. The agglutinogens were prepared by growing the vibrios on Tryptone agar (Difco, Inc.) of pH 7.6, and harvesting them with a minimal amount of 0.9 per cent sodium chloride solution (saline) after 18 to 24 hours incubation at 37°C. This suspension was placed in a boiling water bath for one hour before centrifuging and washing three times with saline. Finally, enough saline was added to make a vibrio suspension matching the sixth tube of MacFarland's nephelometer.

Washed human "O", rabbit and sheep red blood cells were used for adsorption. Enough packed erythyocytes were added to the nephelometrically adjusted vibrio suspension to make a 5 per cent concentration of the red blood cells.

This mixture was incubated in a water bath for 2 hours at 37°C. to adsorb the antigen on the erythrocytes. The red blood cells were then centrifuged and washed three times with saline. The term "adsorbed antigen" is being applied to this agglutinogen which consists of erythrocytes with bacterial cells adsorbed on them. The expression "unadsorbed antigen" is being used to designate the suspension of heat-treated vibrios not adsorbed onto red blood cells. Since sheep cells did not show any advantage over human or rabbit cells, their use was discontinued.

The optimal concentration of the adsorbed antigen for the test proper was established in preliminary experiments. Adsorbed antigens with rabbit and human cells in 2, 1, 0.5 and 0.25 per cent suspensions were tested against increasing dilutions of homologous sera. The mixtures were incubated for one hour in the

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37°C. water bath, then allowed to stand one hour at room temperature before reading. One per cent suspensions of red blood cells, onto which vibrios were adsorbed, gave the most clear-cut results. This concentration was used thereafter in all tests. The volume of adsorbed antigen and of the increasing serum dilutions was kept at 0.25 ml. in each test tube, as in Neter's technic. The serum dilution was 1:20 in the first tube.

The rabbit sera used in these experiments did not show spontaneous agglutination with human or sheep red blood cells. This may occur with other batches of sera and it may be necessary to use nonsensitized heterogenous erythrocytes to absorb hemagglutinins in such instances. Inactivation did not influence the results with rabbit sera but was helpful with sera from vaccinated individuals studied in preliminary tests.

The optimal incubation time was established by experiment. Series of hemagglutination experiments were set up with all three sera and antigens used in this study, and incubated for one and for two hours, respectively, at 37°C. in the water-bath. Then the tubes were allowed to stand at room temperature and read after 30, 60, 90 and 180 minutes. Only tubes with macroscopically visible clumps of red blood cells were considered positive in this and all other experiments. The highest agglutination titers were observed in the series incubated for one hour in the water-bath, followed by 30 to 60 minutes at room temperature.

The question of the necessity of buffering the antigen and the serum was also considered. Employing the three sera and their homologous adsorbed antigens at optimal concentrations and incubation, using buffered saline of pH 5.8, 6.2, 6.6, 7.0, 7.0, 7.4, 7.8 and 8.2, maximal agglutination titers were observed at pH 6.6 to 7.4 in all series. Thus under normal laboratory conditions the use of buffered saline is not necessary in this test.

Adsorbed antigens for the slide hemagglutination experiments were prepared in the same manner as for the serial tube dilution method. Preliminary tests showed that one per cent suspensions of the adsorbed antigens produced the fewest equivocal results.

Serial dilutions of the test sera, beginning with 1:40, were made up. One drop of each dilution was mixed with one drop of the adsorbed antigen in excavations

TABLE 1

Agglutination tests with unadsorbed and adsorbed V. comma *antigens*

Antigen	Agglutination Titer with Homologous "O" Serum in	
	Tube test	Slide test
Inaba, unadsorbed "O"	1280	160
Inaba, adsorbed on RBCs	5120	2560
Ogawa, unadsorbed "O"	1280	80
Ogawa, adsorbed on RBCs	5120	1280
Hikojima, unadsorbed "O"	640	40
Hikojima, adsorbed on RBCs	1280	1280

of agglutination slides. The slides were gently rocked and the results read after 5, 10, 15 and 30 minutes at room temperature. Table 1 shows a comparison between tube and slide agglutinations, using rabbit erythrocytes for the adsorption of the antigens. The titers observed were higher in hemagglutination tests than in experiments with unadsorbed antigens. Visible clumps occurred in slide tests with the homologous antigen rapidly. The agglutination was completed in 15 minutes. Since the titers in slide hemagglutination tests exceeded several times those observed with unadsorbed antigens, the slide method also proved valuable in hemagglutinations with *V. comma*.

SUMMARY

The hemagglutination method, as suggested by Neter *et al.* for enteric infections producing low serum agglutinin titers, was applied to *V. comma* antigens and antisera. Both serial tube dilution and slide tests were employed and gave satisfactory results.

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TREATMENT OF *ENTEROBIUS VERMICULARIS* INFECTIONS WITH PIPERAZINE[1]

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The effectiveness of piperazine against human enterobiasis has been demonstrated by the clinical studies of Mouriquand, Roman and Coisnard (1951), White and Standen (1953) and Bumbalo, Gustina and Oleksiak (1954). A dosage schedule of 7 days' therapy, 7 days' rest, followed by an additional 7 days of therapy was employed by the latter two groups of workers, a therapeutic regime very similar to that used by Wright, Brady and Bozicevich (1938) when they introduced gentian violet for the treatment of pinworm.

Clinical observations in man suggest that adult pinworms are especially susceptible to piperazine, and the studies of Brown, Chan and Hussey (1954) on the pinworm (*Syphacia*) of mice demonstrate conclusively the great efficacy of piperazine against adult worms and its very slight effect on the immature stages. These observations suggested that an interrupted treatment spread over several weeks might be especially effective, as it would allow time for immature forms to develop to the mature, susceptible stage. For the above reasons, in our early therapeutic trials of piperazine against *Enterobius* infections, we also employed the split 14-day course of treatment. The results were very good, but we found a number of these patients with pinworm eggs in their perianal area during the 7-day rest period between the courses of therapy. Spread of infection in the family and self-infection were therefore both possible. Furthermore, it was felt that a shorter therapeutic regimen, if equally effective, would be advantageous. We therefore treated a number of pinworm-infected patients for 10 and 14 consecutive days respectively.

METHODS

One hundred and twenty pinworm-infected individuals, 1 to 52 years of age, were treated in this study. A group of private patients were referred to us by private physicians, including a number of doctors' families. In this group the mother of the family was instructed in the method of utilizing the Scotch tape swab diagnostic preparation, and in the physicians' families the physician himself secured the swab specimens. We used Scotch tape Number 800, which is especially transparent. A number of these patients had been unsuccessfully treated previously with gentian violet, terramycin and egressin. The second group consisted of teen-age and adult patients in a local training school for mental defectives. All swabs on this group were taken by one nurse whom we

[1] Burroughs, Wellcome and Company, Sharp and Dohme, and Winthrop-Stearns, Inc., kindly furnished the piperazine citrate syrup used in these studies. We wish to thank Dr. Elizabeth Nesbitt of the North Jersey Training School for her cooperation in this study and Miss Mary Jani for her assistance in examining the numerous Scotch tape swabs.

instructed in the technique. Due to the poor hygienic habits of this latter group, they were especially vulnerable to reinfection.

The piperazine citrate syrup was measured in a standard teaspoon which held 5.0 cc. when moderately full. The medication was given by the mother to the private patients, and by a nurse to the institution patients. Our experience in the laboratory with this method of liquid measurement demonstrated that the spoon held from 4.0 cc. to 6.0 cc. depending upon how full it was and the height of the meniscus. It is obvious that this method of measurement is open to considerable variation.

The dosage schedule employed was as follows:

Patient's Weight	Piperazine Citrate Syrup	Equivalent Piperazine-hexahydrate
15–30 lbs.	½ teaspoonful (2.5 cc.) b.i.d.	0.25 gm. b.i.d.
31–60 lbs.	1 teaspoonful (5 cc.) b.i.d.	0.5 gm. b.i.d.
61 lbs. and over	2 teaspoonfuls (10 cc.) b.i.d.	1.0 gm. b.i.d.

Seven days after the completion of treatment, a Scotch tape swab preparation was secured from the patient and this procedure continued for 7 consecutive mornings. Any swab showing one or more eggs indicated a failure to cure, while failure to find eggs on all 7 swabs was our criterion for cure.

The parents of the private patients were informed about the usual hygienic precautions against reinfection and many of them put them into effect. It was not practicable to institute these measures in the institutional group.

RESULTS

The 10-day course of treatment eliminated 89 per cent of the *Enterobius* infections, the split 14-day course (7 days' therapy, 7 days' rest, 7 days' therapy), 92 per cent and the 14-day course, 97 per cent (Table 1). Eleven infected individuals were left untreated and examined by the Scotch tape method on 7 consecutive days, along with the treated group. Two of these patients (18 per cent) lost their pinworms during this period without any treatment. It will be noted (Fig. 1) that a total daily dose of 45 mg. per kg. of body weight (hexahydrate) or greater was 100 per cent effective whether given for 10 or 14 days. The treatment failures were in the group receiving 30 to 44 mg. per kg. body weight.

TABLE 1

The effect of piperazine citrate syrup against Enterobius vermicularis *in man*

Dosage Schedule	Patients Treated	Patients Cured	Per Cent Cured
10-day course	57	51	89
Two 7-day courses 1 week apart	24	22	92
14-day course	39	38	97
Untreated controls	11	2	18

RELATIONSHIP OF THE SIZE OF DOSE OF PIPERAZINE HEXAHYDRATE TO ITS EFFECTIVENESS AGAINST PINWORM

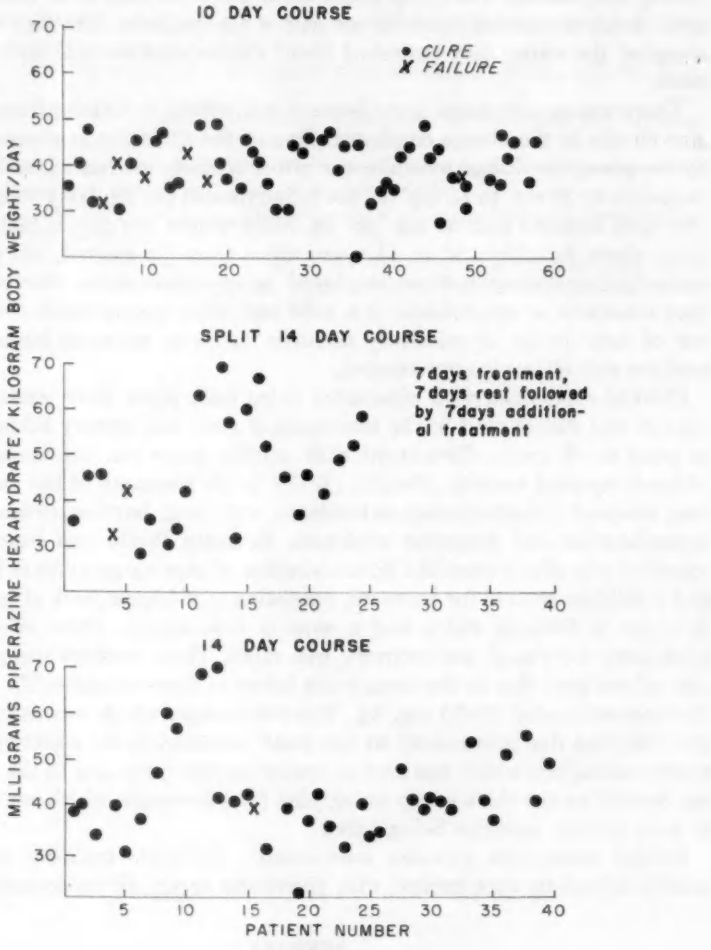
10 DAY COURSE
• CURE
X FAILURE
SPLIT 14 DAY COURSE
7 days treatment, 7 days rest followed by 7 days additional treatment
14 DAY COURSE
MILLIGRAMS PIPERAZINE HEXAHYDRATE / KILOGRAM BODY WEIGHT/DAY
PATIENT NUMBER

FIG. 1

Twenty-six patients who were undergoing the 10-day treatment had a single Scotch tape swab examination on the 7th and 10th days of treatment, and 9 and 5 respectively were found positive. This indicates that many patients who are undergoing the split 14-day treatment will be shedding eggs during the week that no treatment is given between the two 7-day courses. During this period contamination of their environment and transmission of the infection to others as well as auto-infection are possible.

Twelve patients were treated with wafers containing calcium dipiperazine dicitrate employing the same amount of piperazine hexahydrate as was present in the syrup preparation. Ten of the patients, 83 per cent, were cured by this 14-day split course. The syrup preparation was somewhat more effective, as the split 14-day treatment cured 92 per cent of the patients. Although the patients accepted the wafer, they remarked about their somewhat acid and unpalatable taste.

There was no complaint or evidence of side effects or toxicity from the piperazine citrate in the dosage employed. Due to the difficulty in measuring liquids by teaspoon, the dosage schedule was rather coarsely graduated, and daily doses ranged from 22 mg. to 75 mg. (as the hexahydrate) per kg. body weight per day. Our data indicate that 50 mg. per kg. body weight per day is highly effective when given for either 10 or 14 consecutive days. In general, our dosages are somewhat smaller than those employed by several workers. We feel, however, that inasmuch as enterobiasis is a mild and often asymptomatic infection the use of toxic drugs or relatively nontoxic drugs in amounts large enough to produce side effects is unwarranted.

Clinical experience with piperazine dates back some sixty years, and for a time it was widely used in the treatment of gout and urinary lithiasis in doses as great as 10 grams. Treatment with smaller doses was continued for weeks without reported toxicity. Hanzlik (1917), in his summary of the use of piperazine, reported side effects such as headache, vomiting, diarrhea, urticaria, tremor, incoordination and muscular weakness. Recently White and Standen (1953) reported side effects from the administration of piperazine hydrate in 14 adults and 6 children treated for pinworm, consisting of giddiness, lack of coordination, difficulty in focusing vision and a sense of detachment. These effects, for the most part, were mild and recovery was rapid. These workers suggest that the side effects were due to the drug being taken in doses considerably in excess of their recommended 50–75 mg./kg. The overdosage, which was as much as 100 per cent, was due presumably to the great variation in the capacity of the domestic tablespoon which was used in measuring the syrup and to the high meniscus formed by the thick syrup embodying the piperazine, which would also lead to even greater amounts being taken.

Several hookworm (*Necator americanus*), *Trichuris trichiura* and *Giardia lamblia* infections were treated with piperazine syrup, all unsuccessfully.

SUMMARY

Piperazine citrate syrup was used in the treatment of 120 patients harboring *Enterobius vermicularis* with highly effective therapeutic results. Fourteen consecutive days of treatment was more effective than 10 days of therapy or the split 14-day regime consisting of 7 days' treatment, 7 days' rest, followed by another 7 days of therapy. In the dosage employed no side effects or toxicity were encountered.

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THE TREATMENT OF CASES OF ASCARIASIS WITH PIPERAZINE CITRATE[1]

With Observations of the Effect of the Drug on Other Helminthiases

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Fayard (1949) and Mouriquand *et al.* (1951) reported the use of piperazine hexahydrate in the treatment of cases of ascariasis. However, the efficiency of this compound in removing ascarids was not fully evaluated by post-treatment stool examinations and egg counts. Since the start of this study, more thorough evaluations of piperazine citrate (diethylenediamine) for the treatment of cases of ascariasis have been reported by Basnuevo and Alonso Fontao (1953), Basnuevo, Labourdette *et al.* (1953) and by Brown and Sterman (1954).

This paper reports the use of piperazine citrate in the treatment of cases of uncomplicated intestinal ascariasis with light and heavy worm burdens for 3 to 15 days. Observations were made on small children to ascertain their tolerance of piperazine citrate at high dosages for varying periods of time. The only reported evaluation of the treatment of cases of ascariasis with piperazine citrate, in which Stoll dilution egg counts were employed, has dealt in most instances with smaller worm burdens and with lower daily dosage (Brown and Sterman, 1954). Experiences with the use of this drug for the treatment of cases of helminthiases other than ascariasis are also recorded.

METHODS

The following data are based upon observations obtained from the treatment of 26 patients infected with *Ascaris lumbricoides*. The drug product used in this study was an orange-flavored syrup which contained piperazine citrate in an amount equivalent to 100 mg. of piperazine hexahydrate per cc. (Antepar). No pre- or post-treatment purgation and no fasting were employed. Fifteen of the patients were hospitalized. This permitted frequent egg counts on stool specimens and close observation of the children for any possible reactions to therapy. Eleven patients were treated through the hospital out-patient clinic. The mothers were asked to watch for any unfavorable reactions during therapy. Since one group of patients was observed closely in the hospital and the other group was observed by the mothers at home, each served as a check upon the other.

The formalin-ether sedimentation technic was employed on all stool specimens

[1] The piperazine citrate (Antepar) used in this study was provided by Burroughs Wellcome and Company.

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TABLE 1

Treatment of cases of ascariasis with piperazine citrate (Antepar)

No.	Age	Weight (lbs.)	Max. Daily Dosage (mg./lb.)	Days of Therapy	Total Dosage (gms.)	Total dosage (mg./lb.)	Egg Count Before Therapy (per gram.)	Egg Count After Therapy (per gram)	% Reduction
1	3	25	48	15	10	400	117,100	1,900	98
2	2	17	47	10½	8.4	494	12,100	0	100
3	2	31	77	10	16.2	523	42,100	0	100
4	2	27	74	8	15	555	27,000	0	100
5	2	37	54	8	13	351	40,000	0	100
6	2	27	74	7	14	519	35,000	0	100
7	4	38	53	7	14	368	31,200	0	100
8	5	40	68	7	11.4	285	6,700	0	100
9	2	28	32	7	4.3	154	3,000	0	100
10	3	24	25	6	3.6	150	22,400	0	100
11	3	38	53	6	12	316	14,500	0	100
12	3	32	78	5	12.5	391	73,600	0	100
13	3	40	75	5	15	375	13,800	0	100
14	4	33	73	5	7.2	218	3,800	0	100
15	10	60	50	5	15	250	1,500	0	100
16	3	33	76	5	12.5	379	500	0	100
17	2	14	57	4½	3.6	257	14,200	0	100
18	32	118	25	4	12	102	0	0	2 male worms passed
19	5	36	67	4	8.4	233	6,200	0	100
20	11	62	48	3	9	145	31,500	900	97
21	5	49	61	3	9	184	8,500	0	100
22	1	28	71	3	6	214	5,500	0	100
23	5	41	74	3	9	220	5,200	0	100
24	4	40	75	3	9	225	1,500	1,500	0
25	4	34	74	3	7.5	221	1,400	0	100
26	2	21	38	3	2.4	114	900	0	100

before, during and after therapy. Stoll dilution egg counts were made on all stools in which eggs were demonstrable. Stool examinations were done at one to two-day intervals during and after treatment of the hospitalized patients. The examinations continued until the patients were discharged following the failure to demonstrate ova of *A. lumbricoides* in one to six post-treatment stools. Initially, examinations were made of stools of clinic patients at one and two-week intervals after the start of therapy. Later, a single stool specimen was collected one week after treatment was begun. The passage of worms during the treatment period, and the failure to demonstrate eggs in stools collected one and two weeks after the start of therapy, indicated that the infections were being eliminated. The failure to demonstrate eggs after treatment was not due to drug suppression of egg output by the worms.

The egg counts on stools of 25 cases ranged from 500 to 117,000 per gram of feces. Eight patients had egg counts in excess of 30,000 per gram. The daily dosage ranged from 25 to 78 mg. per pound. The anthelmintic was given in two

or three equal doses per day depending upon the volume of drug. Eleven patients received more than 70 mg. of the drug per pound per day. The maximum total dosage per pound over an eight day period was 555 mg. The dosage of approximately 70 mg. per pound was initially selected in view of a statement by White and Standen (1953a) which indicated that they had employed up to 150 mg. of piperazine citrate per kilogram daily in the treatment of ascariasis. However, these workers made no specific mention of their therapeutic results. A summary of the dosages used and the therapeutic results obtained is presented in Table 1. Since there was variation in the amount of drug administered during therapy of some cases, the maximum daily dosage in mg. per pound is indicated.

RESULTS

Therapeutic efficiency. There was 100 per cent reduction of the egg counts in 22 of 25 treated cases. There were egg count reductions of 97 and 98 per cent in two additional cases. In one case with a light infection, there was no reduction of the egg count despite passage of two worms. One case was treated despite the absence of eggs in the stool. A few weeks prior to treatment, the patient's stool had contained fertilized eggs of *A. lumbricoides*. Female ascarids were spontaneously passed and eggs were no longer demonstrable in the stool. Treatment with piperazine citrate resulted in the passage of two male worms.

No treatment failures occurred in patients who received approximately 75 mg. of the drug per pound daily for five days. The total daily dosage did not exceed three grams. Five of seven patients treated for three days with 38 to 75 mg. of drug per pound daily had a 100 per cent reduction in egg counts. However, the worm burdens in the latter group were relatively light. There were no treatment failures in patients who received more than 10 grams of the drug. There was almost complete elimination of *Ascaris* infections in all cases treated for more than three days.

In a few cases, passage of worms was limited to the first or to the initial two days of treatment. The stools of these patients promptly became negative for eggs of *A. lumbricoides*. This indicates that some infections may be eliminated by one or two days of therapy with the dosage employed.

Piperazine citrate effected the elimination of both mature and immature ascarids from the intestine. The worms were still alive when passed during the course of therapy. However, the majority of the worms observed were sluggish or flaccid. It is postulated that the worms had lost their ability to maintain traction against the intestinal mucosa. Immersion of vigorous adult ascarids from human beings in a 1 to 10 aqueous dilution of piperazine citrate syrup resulted in a rapid loss of motility and a flaccid condition of the worms. This action appeared to be reversible since rinsing of the flaccid worms with water resulted in the reappearance of motility.

Tolerance of the drug. Close observation of hospitalized children and interrogation of the parents of children treated on an out-patient basis failed to reveal any side-reactions to therapy. The drug was administered without difficulty or

local effect. Laboratory studies, including BUN, cephalin flocculation, thymol turbidity, urinalysis and complete blood counts, were made on a few patients who received 70 mg. of the drug per pound daily for five days. No abnormal findings were obtained.

One recent case, not included in this series, ingested an estimated 90 to 110 cc. of piperazine citrate syrup. The child was 3 years of age and weighed 23 pounds. A small volume of the drug was vomited five hours after ingestion. Nine hours after taking the medicine a stomach lavage was given. It was estimated that the child ingested about 400 mg. of drug per pound of body weight. The only significant and evanescent findings during the brief hospital stay were the development of hypoactive patellar reflexes and apparent inability to sit erect for a short time. Urine, blood, BUN, and liver profile studies revealed no abnormal findings.

EFFECT OF PIPERAZINE CITRATE ON CERTAIN OTHER HELMINTHIASES

There was an apparent decrease of 83 per cent in the egg count of the stools of one patient with hookworm infection during treatment with 1.5 grams of piperazine citrate daily for 10 days. If further study demonstrates conclusively that this anthelmintic is effective against hookworm, it may be useful in the treatment of mixed infections of *Ascaris lumbricoides*, *Enterobius vermicularis* and hookworm. Ghanem (1953) reported that a complex substituted piperazine compound was active against *Ancylostoma duodenale*.

Piperazine citrate failed to cure three cases of strongyloidiasis (Jones, 1954). The dosage employed was 3 grams per day for 7 to 10 days. One case received six grams of the drug daily for an additional seven days. Larvae of *Strongyloides stercoralis* were demonstrable in the duodenal drainages and intermittently in the stools of all three cases after completion of the treatment.

No significant reductions were observed in the stool egg counts of patients with light *Trichuris trichiura* infections. The trichuriasis was incidental to *Ascaris* infections for which the patients were being treated with a wide range of dosages of piperazine citrate.

Two persons with *Hymenolepis nana* infections were unsuccessfully treated. One case received 2 grams of the drug daily for 5 days; treatment was repeated at a dosage of 3 grams daily for an additional five days. The second case received two seven day courses of piperazine citrate at a daily dosage of 3 grams.

Patients with visceral larva migrans frequently also have *Ascaris lumbricoides* infections. The syndrome of visceral larva migrans appears in patients who apparently are very reactive to the infection. The production of acute allergic or anaphylactic reactions in patients with visceral larva migrans by use of an anthelmintic for ascariasis which might also have some activity on the larvae in the tissues was considered a potential hazard. One patient with both visceral larva migrans and ascariasis and one patient with visceral larva migrans only, were treated with piperazine citrate. No therapy-related reactions were observed in either case during the course of treatment.

DISCUSSION

This study confirms the report by Brown and Sterman (1954) of the high efficacy of piperazine citrate syrup in the treatment of cases of ascariasis. The percentages of reduction of egg counts were very similar in both studies. Also, there was failure to eradicate infection completely in both series of cases with only three days of therapy. The daily dosage of 70 to 75 mg. of drug per pound for four or more days apparently is more than is required to cure most light infections. The apparent tolerance of the above dosage by small children indicates that there is a wide range between the average effective therapeutic dosage and the toxic dosage. Low daily and total dosage may be inadequate for complete cure of very heavy ascarid infections. We are not recommending that daily dosages of 70 mg. per pound be employed routinely. However, in heavy *Ascaris* infections such a dosage was usually completely effective and well tolerated by children in this series of cases.

Basnuevo, Labourdette *et al.* (1953) obtained only a 65 per cent cure rate with a single course of treatment of 20 cases of ascariasis with piperazine. The dosage which they employed was 0.3 gm. of a six per cent solution of piperazine hydrate for each year of age daily for ten days. A second course of piperazine given to the treatment failures increased the percentage of cures to 80.

The treatment of cases of ascariasis with piperazine citrate over a period of several days poses no serious difficulty in the management of individual cases. This would limit its use for mass therapy unless an effective and safe single dose treatment can be obtained.

Brown and Sterman (1954) and Basnuevo and his associates (1953) observed no therapy-related reactions during the treatment of cases of ascariasis with piperazine. Our observations are consistent with their findings. White and Standen (1953b) have discussed intolerance to piperazine hydrate. The reactions which they observed were mild and brief. They may have been related to excessive dosage due to variation in the content of tablespoons. The use of graduated paper cups for measuring the medicine given to patients in this series minimized the possibility of a patient taking excessive medication.

SUMMARY

The efficacy of piperazine citrate (Antepar) for the elimination of light and heavy infections with *Ascaris lumbricoides* over a wide range of dosage was demonstrated. No evidence of intolerance of the drug was observed in children during therapy with high dosage.

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TREATMENT OF WUCHERERIASIS AND ONCHOCERCIASIS WITH SURAMIN SODIUM[1]

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During the course of surveys and various investigations at the Liberian Institute of the American Foundation for Tropical Medicine, it was discovered that 25.6 per cent of the local population examined had onchocerciasis and that 16.4 per cent had wuchereriasis. Even though serious complications from these diseases were rare or absent, treatment was requested by many of the infected individuals. Treatment of these diseases with suramin sodium as used by the writer in Guatemala for onchocerciasis (Burch and Ashburn, 1951) was therefore made available at the Institute.

TABLE 1

Results of examinations for microfilariae in skin biopsies on 17 patients with onchocerciasis treated with suramin

Interval	Number Positive	Per Cent Positive
Pre-treatment	17	100
Post-treatment		
1 to 2 months	7	41
8 to 10 months	2	12

TABLE 2

Average number of microfilariae per 20 cmm. of blood at 9 p.m. on 16 patients with wuchereriasis treated with intravenous suramin

Interval	Microfilariae per 20 Cmm.
Pre-treatment	53
During treatment	65
3 to 4 months post-treatment	29
10 to 13 months post-treatment	40

METHODS

Twenty patients with onchocerciasis and 21 patients with wuchereriasis were treated with from 6 to 9 weekly intravenous injections of suramin. The initial dose was 0.5 gm., and subsequent doses were 1.0 gm. except that the medication was either omitted or given in half dosage in the presence of albuminuria. Total

[1] This work was done as part of the United States Technical Cooperation Administration Program in Liberia and was a collaborative project with the National Public Health Service of Liberia. Laboratory facilities were made available by the Liberian Institute of the American Foundation for Tropical Medicine.

dosage ranged from 3.5 to 7.5 gm. and 0.08 to 0.15 gm. per kilogram. Skin biopsies and scarification smears on all patients with onchocerciasis and thick blood smears using 20 cmm. of blood at 9 p.m. on all patients with wuchereriasis were made before and at various intervals after treatment.

RESULTS AND DISCUSSION

Annoying symptoms during the course of treatment consisting of various aches and pains and chills and fever were very common, but it was difficult to be sure which were due to treatment and which were due to concomitant malaria, intestinal parasitism, etc. Five patients with filariasis developed pain and tenderness in the groin or scrotum during treatment. One of these developed acute lymphangitis of the scrotum after the completion of treatment. All had had previous similar attacks. It is interesting to note that none of the patients developed hyperesthesia of the soles of the feet such as occurred so very commonly in patients treated in Guatemala.

Seventeen patients with onchocerciasis have had periodic follow-up examinations for 8 to 10 months. Approximately half had positive skin biopsies shortly after completion of treatment, but as shown in Table 1 only two or 12 per cent were positive 8 to 10 months later. This is comparable to the results obtained in Guatemala.

All patients with wuchereriasis who were examined approximately one year after completion of treatment had demonstrable microfilariae. The average number of microfilariae per 20 cmm. of blood taken at 9 p.m. was considerably reduced following treatment but then gradually increased as shown in Table 2. At the end of the examination period, however, it was still below the initial level even though half of the patients had microfilaria counts as high as, or higher than before treatment. This would suggest that intravenous suramin in the dosages used has a toxic but not lethal effect against the adult of *Wuchereria bancrofti*.

CONCLUSIONS

Intravenous suramin sodium is effective in treating African onchocerciasis and has a toxic but probably not lethal effect against the adult of *Wuchereria bancrofti*.

ACKNOWLEDGMENT

The co-operation of Dr. Max J. Miller, Director, Liberian Institute of the American Foundation for Tropical Medicine is acknowledged with thanks.

REFERENCE

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CUTANEOUS MYIASIS OF THE SCALP DUE TO *DERMATOBIA HOMINIS* (LINNAEUS JR.) (DIPTERA; CUTEREBRIDAE)

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National Institutes of Health, National Microbiological Institute, Laboratory of Tropical Diseases, Bethesda, Maryland

On April 13, 1953, during the course of onchocerciasis investigations in Guatemala, a man arrived at the laboratory requesting the removal from his scalp of an annoying "filaria" which he said was "ripe". The term "filaria" is commonly used by the people of the endemic onchocerciasis zone for the nodule formed as an inflammatory reaction to the presence of the adult filarid worms. Although he had been experiencing intermittent pain, he complained more of the purulent discharge.

The pustular nodule was located on the left occipital region just posterior to the lambda. It appeared, when palpated, to be easily movable in the connective tissue, thus similar to an onchocercoma. Upon closer examination, after clipping the hair in the general region, movement within the boil could be discerned and the posterior spiracular plates of a maggot were visible at the opening from which the pus exuded. Attempts to remove the maggot by pressure or by plugging the opening to cut off respiration of the larva were unsuccessful. Removal by forceps was also impossible, even after using a local anesthetic and killing the maggot with a few drops of creolin. The difficulty was probably due to the "bottle" shape of the larva, which was situated in the nodule with its narrow end toward the outside. A small incision was made to enlarge the opening and the maggot was then easily removed. The cyst, which was quite extensive, was cleaned and merthiolate only was applied. No sequelae developed.

The larva was definitely identified as *Dermatobia hominis* (Linnaeus Jr.), the human or Neotropical botfly. *D. hominis* commonly causes cutaneous myiasis of cattle as well as of other domestic and wild animals throughout most of Central and South America and parts of Mexico. The female usually oviposits on the abdomen of biting Diptera, primarily day-flying mosquitoes of the genus *Psorophora*, stable flies (*Stomoxys*), and tabanids, and at times on nonbiting flies and ticks. The eggs are so arranged that the operculate end is away from the abdomen of the carrier. When the latter bites or visits an appropriate host, the larvae burst out of the chorion, entering the skin of the host either through the puncture made by the biting carriers, or by penetrating the unbroken skin through a hair follicle, fold, or some other convenient portal of entry.

In Guatemala, this fly has been reported primarily from the lowland regions, where it attacks cattle, wild animals, and only on rare occasions man. The human cases recorded have all been in the arms or legs (Cockerell, 1914; Sticker, 1928). The infested person mentioned above was about 25 years of age and of Mayan

[1] The author wishes to express his thanks to Dr. Maurice T. James for having made available his bibliography of *D. hominis*.

Indian stock. He worked in the coffee fields of the Finca San Lucas Miramar, Yepocapa, Chimaltenango, located at approximately 3,300 feet above sea level on the Pacific slope of the Volcano Acatenango. This plantation is at the lower limit of a semitropical rain forest and at the upper limit of a coastal cattle-raising region. In considering possible arthropod carriers of *Dermatobia* eggs in this area, it is believed unlikely that mosquitoes are involved since they are only infrequently encountered. Examination of thousands of black flies (*Simuliidae*) and punkies (*Heleidae*) relative to onchocerciasis studies never disclosed members of these families with attached *Dermatobia* eggs. Probably tabanids, some of the muscoid Diptera, or ticks actually serve this function.

The reported cases of myiasis of the head due to *Dermatobia hominis* are few in comparison to those of other body regions. Palpebral myiasis has been reported from Brazil (Fisher, 1922) and Panama (De Cerqueira, 1927), and a single, very striking case of cerebral myiasis in Panama has been discussed by Dunn (1934). In regard to the latter, the larvae penetrated through the scalp and at least one passed through the fibrous portion of the anterior fontanel of a one and one-half year old child, entering the left hemisphere of the brain. The child suffered convulsions and finally died. It is doubtful that penetration could occur through the bony skull of an adult.

SUMMARY

Report is given of a case of cutaneous myiasis of the scalp due to the Neotropical botfly, *Dermatobia hominis* (Linnaeus Jr.) in Guatemala. An incision had to be made to enlarge the opening of the boil-like nodule before the larva could be removed.

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TRANSACTIONS OF THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

Third Annual Meeting

Memphis, Tennessee, November 3–6, 1954

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Herbert C. Clark 1936–1937
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Alfred C. Reed (deceased) 1938–1939
Louis L. Williams, Jr. 1939–1940
Thomas T. Mackie 1940–1941
Ernest Carroll Faust 1941–1942
N. Paul Hudson 1942–1943
Wilbur A. Sawyer (deceased) 1943–1944
Rolla E. Dyer 1944–1945
James S. Simmons (deceased) 1945–1946
Edward I. Salisbury 1946–1947
Joseph S. D'Antoni 1947–1948
Norman H. Topping 1948–1949
Hamilton H. Anderson 1949–1950
Paul F. Russell 1950–1951
William A. Sodeman 1951–1952

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H. A. Johnson 1944–1945
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Mark D. Hollis 1946–1947
E. Harold Hinman 1947–1948
Wendell Gingrich 1948–1949
Paul F. Russell 1949–1950
Justin M. Andrews 1950–1951
W. H. W. Komp 1951–1952

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Martin D. Young 1952
William A. Sodeman 1952–1953

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Representative to the American Foundation for Tropical Medicine: E. I. SALISBURY.

Representative to the National Research Council: H. MOST (three years).

Representative to the Kimble Methodology Research Award Nominating Committee: E. C. FAUST.

Representative to the Steering Committee, Proposed American Board of Medical Microbiology: A. S. ALVING.

MINUTES OF THE ANNUAL COUNCIL MEETINGS

The annual business meetings of the Officers and Councilors were held at the Hotel Peabody, and were presided over by the President, Frederick J. Brady. The first meeting was held Wednesday morning, November 3, and the final meeting was held that evening. Officers and Councilors present were: William H. Taliaferro, Lewis W. Hackett, Thomas F. Whayne, Herbert G. Johnstone, G. Robert Coatney, Clay G. Huff, Samuel W. Simmons, Albert B. Sabin, and James J. Sapero. Past-Presidents of the parent Societies, and the present Society, in attendance were: Hamilton H. Anderson, George H. Bradley, George R. Callender, E. Harold Hinman, Edward I. Salisbury, and Martin D. Young. Dr. George R. Callender and Dr. E. Harold Hinman represented the American Academy of Tropical Medicine.

The minutes of the second meeting held November 12–13, 1953 in Louisville, Kentucky were accepted as published in the May, 1954 issue of the JOURNAL.

The Secretary's report was presented as follows:

This report covers the period from November 1, 1953 to October 31, 1954. Highlights of the year were: (1) the Gorgas and WHO resolutions, (2) reappointment of Dr. Lewis W. Hackett as Editor-in-Chief of Society publications, (3) change in conditions for the Joseph Augustin LePrince Award, (4) consideration of changes in the Constitution and By-Laws to provide for FELLOWS, (5) new membership list, and (6) arrangements for the annual meeting. Other items of interest are included by number (7–9) below.

(1) *The Gorgas and WHO Resolutions.*—These were included in the report of the Resolutions Committee at Louisville last Fall (see the Transactions, May, 1954 issue of the JOURNAL). Soon after the meetings, copies of the respective documents were sent to various leaders in the Government, and to certain professional organizations and individuals.

(2) *Reappointment of Dr. Hackett as Editor-in-Chief of Society Publications.*—By a unanimous mail vote of the Council (May 18), Dr. Hackett was elected to remain in office after

expiration of his present term on December 31, 1954. The term of appointment is for 3 years.

(3) *Change in Conditions for the Joseph Augustin LePrince Award.*—In a mail ballot dated May 18, the Council voted to include a bronze medal for this Award (see the June, 1954 issue of the NEWS for an account of this Award and a complete list of the conditions).

(4) *Consideration of Changes in the Constitution and By-Laws to Provide for* FELLOWS.—In the report of the Academy Liaison Committee at Louisville last Fall (see Transactions, May, 1954 issue of the JOURNAL), it was suggested that we consider having FELLOWS among our members. On two occasions (November 24, 1953, and August 23, 1954), the Council voted by mail in favor of amendments necessary to include this new class of members. Accordingly, a mail ballot was sent to all voting members on September 14 with a request to vote on the proposed amendment of the Constitution. An overwhelming majority (335 of 363 ballots received) voted in favor of the amendment, which opens the way for consideration of the proposed amendment of the By-Laws. This amendment, which provides for the election of FELLOWS, will be voted upon by the members at the business meeting Saturday morning.

(5) *New Membership List.*—During its meeting at Louisville, the Editorial Board voted in favor of publishing a membership list biennially. The last list, covering 1952, was published in January, 1953. Thus, a new list for 1954 will be published in January, 1955.

(6) *Annual Meeting.*—Early in March, the Officers and Councilors gave approval to a suggestion by many members in the past that the meeting be opened on Wednesday afternoon with adjournment Saturday noon, and they selected the topic and moderator for the symposium to be sponsored jointly with the American Society of Parasitologists. The announcement of the meeting, including the annual call for papers and a ballot for election of certain Officers and Councilors, was mailed to each member on April 10. From that time until the opening of the meeting, the necessary arrangements were made by correspondence with the Local Committee, and various other committees and individuals.

(7) *Membership.*—As of October 31, 1954, the Society had 1070 active members in good standing. Also, the rolls include 17 Honorary Members, 6 Emeritus Members, and 1 Life Member (Dr. Charles A. Werner designated a Life Member on April 19, 1954) making a grand total of 1094 members in good standing. In addition, there are 87 active members, delinquent in dues for 1953 and/or 1954, who failed during the past year to respond to statements of dues sent on three occasions. As per Article X of our By-Laws, only members in arrears for three years are dropped from the rolls. Therefore, with 1094 members in good standing, and 87 members in arrears for 1 or 2 years, we have a total membership of 1181.

Included in the above total of 1070 active members in good standing are 58 new members elected after publication of the 1953 Transactions. The new members are as follows:

ARBUTHNOT, J. B.
BENENSON, A. S.
BUCHANAN, W. J.
BURTON, G. J.
BUSTOS, J.
CALVERLEY, E. T.
CARMICHAEL, D. B.
CHENG, T. C.
CLAPP, R. L.
DEHNÉ, E. J.
DELAMATER, E. D.
DOBBIN, J. E., JR.
DUNN, M. C.
FATTAH, M. A.
FELDMAN, H. L.
FITE, F. W.
GARCIA-LAVERDE, A
GARRIDO LECCA, G.
GOLDMAN, M.
GORDON, I.
HERBERT, A.
HOPPER, S. H.
IRALU, C.
IVEY, M. H.
JAROSLOW, B. N.
JOHNSON, H. N.
KALLINIKOS, G. G.
KIM, C. W.
KNISELY, M. H.
KOLLERT, W. F.
KUHN, E. F.
LEMON, F. R.
LUCASSE, C.
LYNCH, J. E.

LYSENKO, M. G.
MCCABE, L. J., JR.
MANNS, M. M.
MARSHALL, I. H.
MILLER, J. H.
NAJJAR, A. E.
NELSON, T. L., JR.
NICHOLS, R. L.
OSSI, G. T.
PEABODY, G. E.
PRICE, D. L.
PRICE, R. D.
PRINDLE, R. A.
PURSHOTTAM, N.
SCHINAZI, L. A.
SCHUMACHER, R. J.
SENTERFIT, L. B.
SIEGEL, G. S.
SOUTHERN, I. W.
STERMAN, M. M.
TORPY, T. F.
TWOHY, D. W.
WAGSHOL, L. E.
WHITE, P. C., JR.

The Secretary announces with regret the loss by death of five active members:

CORNELL, V.
DUNHAM, G. C.
MILLS, M. A.
SIMMONS, J. S. (Past-President of the NMS, 1942–43; Past-President of the ASTM, 1945–46).
WOLBACH, S. B.

The following submitted resignations:

ABINANTI, F. R.
ANGRIST, A. A.
BELTRAN, E.
BUSSART, J. E.
DAMON, S. R.
DERIVAS, D.
EMMONS, C. W.
FOORD, A. G.
GILBERTSON, W. E.
GREENSLIT, F. S.
HARGETT, M. V.
HATHAWAY, E. S.
HORNER, W. H.
KAUNG, D. T.
KNIGHT, K. L.
MANGUN, C. W.
MUGRAGE, E. R.
MUSCHEL, L. H.
NAILON, W. T.
OLSON, B. J.
PAYNE, G. C.
RIGDON, R. H.
SIMPSON, M. L.
WEINSTEIN, J.
WILLIAMS, C. L.

(8) *Letters to the Officers and Councilors.*—Five letters were mailed during the period of this report. The first letter, November 24, was sent at the request of the Council of the American Academy of Tropical Medicine. At the Louisville meetings, the Academy had accepted a resolution favoring amalgamation with the Society, but it had not been possible at the meetings to obtain the reaction of the Society Council. Thus, the mail ballot of November 24 concerned the reaction to the principle of amalgamation of the Academy and the Society. In addition, the Council was requested to vote on the desirability of a Constitutional Amendment to provide for FELLOWS in our Society. Both the principle of amalgamation and the desirability of an amendment were approved by a large majority. On March 5, a letter was sent concerning tentative plans for the annual meeting and various items were submitted for vote. The results, based on a majority vote, were as follows: (1) the suggestion of opening the meeting on Wednesday afternoon with adjournment Saturday noon was approved, (2) it was voted to schedule two periods for Council meetings (one 9:00 A.M. Wednesday, the other that evening), and it was decided by vote not to arrange a dinner meeting, (3) the suggestion of holding a joint symposium with the American Society of Parasitologists was approved, and the topic ("Schistosomiasis") and moderator (Dr. Donald B. McMullen) were chosen, and (4) the dates of November 2–5 were approved for the 1955 meeting, provided that there is not a conflict with the APHA meeting. On May 18, the following items were submitted for consideration and vote: (1) investment of our surplus in Series K Government Bonds, and using the interest only for reviews in the JOURNAL

(the balance needed for reviews to be taken from the JOURNAL profit for the current year), (2) the investment of the life membership fee of Dr. Charles A. Werner in Series J Government Bonds, (3) the transfer of Dr. J. E. Ackert from active to emeritus membership, (4) change in the conditions of the J. A. LePrince Award to include a bronze medal (and authorization to use Society funds to cover the cost of the medal and book of certificates), and (5) the reappointment of Dr. Hackett as Editor of Society publications. Items 1–4 were approved by a large majority, and item 5, as expected, was approved unanimously. On August 23, the fourth letter was sent and it contained three items for consideration: (1) a document by Dr. Albert B. Sabin concerning "some thoughts on the establishment of a commission to evaluate the medical and hygienic problems in the future development of the tropics and to make recommendations for the future activities of the U. S. A.," (2) the desirability of a resolution concerning the promotion of tropical medicine—this, if approved, to be sent to certain leaders in the Government, and (3) the recommendations of the Society Organization Committee for amending the Constitution and By-Laws to provide for FELLOWS. A vote on item 1 was not requested, since it had been placed on the Council agenda for the Memphis meetings. Items 2 and 3 were approved by a unanimous vote. On October 15, the fifth and final letter was sent. This concerned certain plans for the annual meeting and a request to submit last-minute items for Council consideration at Memphis.

(9) *Other Activities.*—Those in connection with various committees will be presented below under the appropriate headings.

Finally, the fine cooperation of the Officers, Councilors, members of Committees and others in transacting the business of the Society is hereby acknowledged with sincere thanks and appreciation.

Respectfully submitted,
JOHN E. LARSH, JR.
Secretary

The report of the Secretary was accepted as read.

The report of the Editor of the AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE was presented as follows:

Our Journal completes its third volume in 1954, which (with the November issue now in press) will total approximately 1130 pages. This is not quite as large as the all-time record of 1166 pages in 1953, when for some unexplained reason there was an extraordinary affluence of manuscripts, but it is as large as can conveniently be bound in one volume at the end of the year. The Publishers have remarked that, as compared with scientific journals in general, it provides well above the average number of articles for the subscription dollar. The Medical Library Association reports (JAMA, Feb. 20, 1954) that the average subscription rate of 75 medical journals is $6.60 and the cost per page to subscribers is 0.93 cents per page, as compared with our cost in 1953 of about 0.52 cents per page to members of the Society, and 0.53 cents in 1954.

The printing cost per page of text has gone up from $12.35 in 1952 to $13.84 in 1953, and advertising revenue has decreased by about 20 per cent. This has reduced the surplus returned to our Society by the publishers from $3,030.27 in 1952, to $2,693.55 in 1953, in spite of an increase in income from a larger membership. We are still, however, in a very sound financial situation with ample margin to confront the future with serenity (Table 1).

The slight reduction in the size of Volume 3, 1954, is due not so much to financial considerations as to a falling off in the number of manuscripts submitted. The figures through September as compared with previous years are as follows:

Through Sept.	*Ms. rec'd.*	*Accepted*	*Declined or withdrawn*
1952	107	81 (76%)	26
1953	125	104 (83%)	21
1954	99	83 (84%)	14

TABLE 1

Financial statement for Volumes 1 and 2, American Journal of Tropical Medicine and Hygiene

	Vol. 1 (1952)	Vol. 2 (1953)
Income:		
Paid subscriptions	$18,604.00	$18,961.28
Less 72 pages transferred to reserve	1,488.32	
Credit for 42 pages transferred from reserve		868.14
Total from subscriptions	17,115.68	19,829.42
Space sales for advertising	4,212.63	3,404.37
Paid by contributors for extra pages	3,339.78	3,193.68
Net from reprint sales	72.45	153.61
Back volume sales	425.44	513.39
Total income	25,165.98	27,094.47
Production cost:		
Printing of text	13,241.15	16,219.02
Printing cost of advertising section	1,795.57	1,266.63
Distribution, overhead, etc.	4,068.72	4,221.72
Total cost	19,105.44	21,707.37
Surplus:	6,060.54	5,387.10
Accruing to Am. Soc. Trop. Med. Hyg. (50%)	3,030.27	2,693.55
Circulation and size:		
Number of paid subscriptions		
Members	1,071	1,105
Nonmembers	1,282	1,298
Total subscriptions	2,353	2,403
Pages		
Text	1,072	1,166
Advertising section	156	112
Total pages	1,228	1,278

TABLE 2

Number and disposal of manuscripts submitted

Manuscripts	Vol. 1 (1952)		Vol. 2 (1953)	
From previous year		65		63
Submitted during year	148		177	
Declined	23		33	
Withdrawn	9		5	
Accepted		116		139
Available for publication		181		202
Published during year		118		112
On hand for next volume		63		90

However, 1953, which must be regarded as an exceptional year, produced a surplus of 90 accepted manuscripts for which there was not room in the Journal during that year and which provided an unusually large backlog of papers for 1954 (Table 2). The annual meeting of the Society generally produces a spate of manuscripts in the last quarter, enough for several numbers, which puts the Editor in the strong position of being able to decline the mediocre as well as the bad papers.

The amount paid by authors or their institutions for extra pages to swell our volumes has also fallen off:

	Extra pages	*Per cent of total pages*	*Amount rec'd.*
Published in 1952	244	23	$3,339.78
1953	232	20	$3,193.68
1954	182	16	$2,079.06

This is the income which enables us to publish numbers larger than the contractual 150 pages guaranteed by the publisher. We therefore anticipate an excess of approximately 25 pages in 1954 not covered by our contract and the subventions from authors. The resulting deficit, estimated at about $350, has been intentionally incurred in order to keep pace with the inflowing manuscripts, and is covered by a reserve fund of $2000 which stands to the credit of the Journal on the books of the Society.

The fields covered by the papers published during the last two years are shown in Table 3. It will be seen that although nutritional deficiencies bulk large in any picture of tropical disease and hygiene, we received practically no papers at all in that field. The Editor has solicited and obtained two articles on nutrition, one of which will appear in November. He can only imagine that the numerous workers in this field do not consider our Journal sufficiently specialized to use for communication of their results. They appear to be satisfied to write for each other.

Respectfully submitted,
L. W. HACKETT
Editor

The Editor's report was accepted as read. There was a discussion concerning the loss after this year of the account of one of the Companies using advertising space in the JOURNAL. It was decided to call to the attention of members of the Society the importance of patronizing our advertisers and mentioning the JOURNAL when discussing orders, etc. with their agents. The Council was in agreement

TABLE 3

Subjects and approach of papers published by the Journal in 1953 and 1954

Subjects	1953	1954	Approach	1953	1954
Animal hosts and vectors	23	21	Biology and taxonomy	9	4
Bacteria and fungi	9	13	Clinical (diagnosis and treatment)	31	37
Deficiencies and nutrition	0	1	Epidemiology and statistics	23	39
Helminths	16	26	Experimental—field	5	0
Protozoa	50	48	Experimental—laboratory	30	30
Viruses and rickettsias	7	13	Prevention and control	11	12
General and miscellaneous	7	8	Others	3	8
Total	112	130	Total	112	130

that the abstracts of papers on the program should be published with the Transactions as part of the record of the meeting. This matter was referred to the Editorial Board. (*Note:* the Editorial Board voted later in favor of this suggestion, and the plan will be initiated next year. Realizing that certain authors may not wish to have their abstracts included in the published group it was decided to leave the decision to the individuals involved).

The report of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS was presented as follows:

The mailing list of TROPICAL MEDICINE AND HYGIENE NEWS remained approximately the same during 1954. Approximately 2200 copies have been distributed at each mailing.

We are greatly indebted to our advertisers, Burroughs Wellcome and Co., Charles Pfizer and Co., Winthrop-Stearns Inc., and the United Fruit Co. for their financial support. Unfortunately, we lost the patronage of one advertiser during the year, but we have been able to maintain publication because we have also received support from the American Foundation for Tropical Medicine. The Foundation has borne part of the cost of printing and mailing in return for distribution of the NEWS to its mailing list.

The only major change in production of the NEWS was the adoption of a slightly different page size. The page has been reduced from 6 by 9 inches to 5½ by 8½ inches in order to allow the use of a heavy enamelled paper. Because of mechanical and economic considerations it would not have been possible to use the better paper without this change. We feel justified in having adopted the photo offset method of production because of our experience with it during the year. In the case of most issues there has been a delay of less than two weeks between the preparation of copy and production and mailing. The October issue was prepared and printed in one week.

In order to avoid appearance of the JOURNAL and the NEWS at approximately the same time, we have altered the time of appearance of the NEWS to the latter part of even-numbered months. Previously, our schedule called for publication on the first of the even-numbered months, which coincided with the schedule of the JOURNAL calling for publication at the end of the odd-numbered months.

An additional printing of the supplement "Health Hints for the Tropics" was again necessary. Seven thousand copies were produced and the majority of these have already been sold. The cost of this printing has already been paid by receipts from the sales.

A number of individuals in foreign lands have responded to our request, published in an early issue of the NEWS, for correspondents. As soon as a few more are appointed, a list of our correspondents will be published in the NEWS. We are grateful to the members who have sent us newsworthy items and we wish to encourage all members to let us know of their activities and of special events with which they are familiar.

Respectfully submitted,
LEON JACOBS
Editor

This report was accepted as read, and the Council agreed that the policies concerning the Editor's office, i.e., funds for secretarial assistance, etc., should remain unchanged from those agreed upon at last year's meeting.

The Treasurer's report was presented as follows:

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

BALANCE SHEET

September 30, 1954 *Exhibit A*

Assets

Current Assets:			
Cash in bank and on hand ($1.35)—Exhibit B			$2,245.17
Interest receivable, U. S. Savings Bonds, Series K, Schedule 1			179.40
Total Current Assets			$2,424.57
Investments:			
Tropical Medicine and Hygiene News—Exhibit C		$1,989.29	
U. S. Savings Bonds, at cost, Schedule 1:			
Series J, maturity value $275.00	$198.00		
Series K, 2.76%	20,000.00		
Total U. S. Savings Bonds—Note 1		20,198.00	22,187.29
Office Equipment			439.58
Total Assets			$25,051.44

Liabilities and Net Worth

Current Liabilities—Note 2			
Net Worth:			
Investment in office equipment			439.58
Retained earnings:			
Balance, October 31, 1953		$13,333.57	
Increase in retained earnings for the eleven months ended September 30, 1954:			
Ordinary increase in retained earnings U. S. Savings Bonds, Series F, redemption value $8,800.00 from dissolved American Society of Tropical Medicine, with a cost value of $6,512.00 were redeemed for $7,914.50, the proceeds of which were used to purchase U. S. Savings Bonds, Series K	$3,363.79		
	7,914.50	11,278.29	
Balance, September 30, 1954			24,611.86
Total Liabilities (Note 2) and Net Worth (Note 3)			$25,051.44

Notes:

1. Interest on Series J is available for current expenditures, and on Series K for reviews in the Journal.

2. The 1954 editorial office expenses for the nine months ended September 30, 1954 and the September, 1954 salary for secretarial assistance in the office of the Secretary-Treasurer are not included in the Balance Sheet or the accompanying Cash Receipts and Disbursements Statements.

3. In 1954 the Williams and Wilkins Company submitted a statement of operations for the year 1953 for the publishing of the Journal, remitting $2,693.55 as 50% of the profit

for the year 1953. The above Balance Sheet Statement or the accompanying Statement of Cash Receipts and Disbursements, Exhibits A and B, do not reflect 50% of any profit or loss for the nine months ended September 30, 1954 for the publishing of the Journal.

4. Of the $24,611.86 retained earnings, $2,413.86 is unappropriated at September 30, 1954, the remainder having been appropriated for:

American Journal of Tropical Medicine and Hygiene for extra pages	$2,000.00
Life membership fee of Dr. Charles A. Werner invested in U. S. Savings Bonds, Series J	198.00
Investment in U. S. Savings Bonds, Series K, the interest on which is available for reviews in the Journal	20,000.00
Total Appropriated Retained Earnings	$22,198.00

The American Society of Tropical Medicine and Hygiene
Chapel Hill, North Carolina

INVESTMENT IN U. S. SAVINGS BONDS — *Exhibit A*

September 30, 1954 — *Schedule 1*

Serial Numbers	*Date of Purchase*	*Cost*	*Maturity Value*	*Accrued Interest*
Series J:				
Q 21,413J	6-29-54	$18.00	$25.00	
Q 21,414J	6-29-54	18.00	25.00	
Q 21,415J	6-29-54	18.00	25.00	
Q 93,631J	6-29-54	72.00	100.00	
Q 93,632J	6-29-54	72.00	100.00	
Total Values of Series J		$198.00	$275.00	
Series K:				
M 91,038K	4-14-53	1,000.00	1,000.00	$13.80
M 91,039K	4-14-53	1,000.00	1,000.00	13.80
M 91,040K	4-14-53	1,000.00	1,000.00	13.80
M 91,041K	4-14-53	1,000.00	1,000.00	13.80
M 195,142K	6-29-54	1,000.00	1,000.00	9.20
V 26,490K	6-29-54	5,000.00	5,000.00	46.00
V 26,514K	7-9-54	5,000.00	5,000.00	34.50
V 26,515K	7-9-54	5,000.00	5,000.00	34.50
Total Values of Series K (2.76%)		$20,000.00	$20,000.00	$179.40
Total Value of Investments		$20,198.00	$20,275.00	$179.40

Notes:

All bonds are in the name of The American Society of Tropical Medicine and Hygiene, a corporation, in care of School of Public Health, Chapel Hill, North Carolina.

The Series J bonds were purchased with funds from the life membership of Dr. Charles A. Werner. The interest from these bonds is available for current expenditures. Interest accrues to the value of the bonds periodically in accordance with the length of time held.

The Series K bonds were purchased with funds from current operations $6,027.50, reinvestment of $2,016.00 Series J redeemed for $2,058.00, and reinvestment of $6,512.00 Series F from the dissolved American Society of Tropical Medicine redeemed for $7,914.50. The total purchases of Series K bonds in 1954 were $16,000.00. Interest on all Series K bonds is available for reviews in the Journal.

Statement of Cash Receipts and Disbursements

(Eleven Months Ended September 30, 1954) *Exhibit B*

Cash Balances, October 31, 1953:			
Bank of Chapel Hill			$7,302.26
On hand			15.31
Total Cash Balances, October 31, 1953			$7,317.57
Receipts:			
1954 Membership dues, net of refunds		$4,616.25	
1953 Membership dues		40.00	
Proceeds from sale of U. S. Savings Bonds:			
Series F at cost $6,512.00	$7,914.50		
Series J at cost $2,016.00	2,058.00	9,972.50	
The American Journal of Tropical Medicine and Hygiene, profit for calendar year 1953		2,693.55	
Publication of Tropical Medicine and Hygiene News—Exhibits C and D		1,000.00	
Annual meeting, Louisville, Kentucky:			
Share of income from commercial exhibits	688.35		
Balance from Local Committee on Arrangements for registrations, et cetera	276.30		
Refund of advance to Local Committee on Arrangements for annual meeting	100.00	1,064.65	
LePrince Award, honorarium provided by Michigan Chemical Corporation		500.00	
Life membership fee of Dr. Charles A. Werner		192.00	
Refund on paper by Dobrovolny *et al.*		83.45	
Interest on $4,000 U. S. Savings Bonds, Series K		55.20	
Commemorative medal sales		46.75	20,264.35
			$27,581.92

Disbursements:

Office Expenses:

	Editorial	*Sec.-Treas.*	
Secretarial assistance	$579.75	$2,110.00	$2,689.75
Travel and meetings	332.97	343.25	676.22
Office equipment	33.20	406.38	439.58
Postage	121.71	292.42	414.13
Printing	27.44	641.00	668.44
Office supplies	17.39	30.69	48.08
Telephone and telegraph	31.45	7.88	39.33
Legal and auditing fees	—	183.50	183.50
Die changes on Reed Medal	—	148.00	148.00
Advance for 1954 annual meeting	—	100.00	100.00
Freight, N. M. S. Journals	—	78.20	78.20
Corporate registration and filing fees	—	30.00	30.00
Contribution to National Society for Medical Research	—	25.00	25.00
Miscellaneous	—	38.27	38.27
Total Office Expenses	$1,143.91	$4,434.59	$5,578.50

Subscriptions to the Journal		3,458.00	
Geiman's prints for historical paper		18.80	
Purchase of U. S. Savings Bonds:			
Series J, Maturity Value $275.00	198.00		
Series K	16,000.00	16,198.00	
Payment to U. S. Treasurer on paper by Dobrovolny *et al*		83.45	25,336.75
Cash Balances, September 30, 1954—Exhibit A			$2,245.17
Consisting of:			
Cash in Bank of Chapel Hill, Chapel Hill, N. C.			$2,243.82
Cash on hand			1.35
Total Cash in Bank and on Hand			$2,245.17

Tropical Medicine and Hygiene News
Bethesda, Maryland

BALANCE SHEET

September 30, 1954 — *Exhibit C*

Assets

Current Assets:	
Cash in bank—Exhibit D	$1,833.27
Account receivable—American Foundation for Tropical Medicine	103.00
Postal deposit on metered mail	53.02
Total Assets—All Current Assets	$1,989.29

Net Worth

Retained Earnings:		
Balance, November 12, 1953	$2,841.54	
Increase in retained earnings for period	147.75	
	2,989.29	
Payment to The American Society of Tropical Medicine and Hygiene—Exhibits B and D	1,000.00	
Balance, September 30, 1954		1,989.29
Total Net Worth—Exhibit A		$1,989.29

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS

November 12, 1953 through September 30, 1954 — *Exhibit D*

Cash Balance, November 12, 1953		$2,311.09
Receipts:		
Advertising	$2,125.00	
American Foundation of Tropical Medicine	530.48	
Sale of supplement	295.50	2,950.98
		$5,262.07

Disbursements:		
Payment to The American Society of Tropical Medicine and Hygiene—Exhibits B and C	1,000.00	
Printing of News	792.60	
Copy preparation	383.30	
Secretarial assistance	450.00	
Reprinting of Health Hints	351.55	
Stationery and printing	126.59	
Postage meter deposits	145.00	
Postage and express	114.76	
Travel	65.00	3,428.80
Cash Balance, September 30, 1954—Exhibit C		$1,833.27
Consisting of:		
Cash in Bank of Bethesda, Bethesda, Maryland		$1,833.27

By permission of the Council, the accounts were closed September 30th in lieu of the usual closing date of October 31st. The previous date presented a hardship, because of the brief period prior to the meetings during which time the accounts, etc. had to be completed for presentation to the C. P. A. and the Treasurer's report had to be prepared. Because of the earlier closing date, the report this year covers an 11-month period.

First, it will be noted that the sheets prepared by the C. P. A. differ somewhat from those submitted in previous years. The present style, with exhibits, notes, and a schedule, was suggested in an effort to present a more comprehensive report of our financial status. It will be noted that the sheets include as new items a complete listing of our investments (Exhibit A), an itemized account of office expenses of the Editor (Exhibit B), and a complete analysis of the NEWS accounts (Exhibits C and D). It is felt that the present report, with these inclusions, satisfies the directives to the Treasurer (Article IV of our By-Laws).

To clarify the situation in regard to an item in last year's report, it seems wise to consider first our Government Bond holdings. At the time of the Louisville meetings last Fall, the bonds ($6,512.00 at cost) transferred from the American Society of Tropical Medicine had not been reissued in the name of the new Society, although application for reissue along with evidence of ownership had been filed many months previously with the Federal Reserve Bank of Richmond. To satisfy the Treasury Department as to our claim to the bonds, it was necessary to secure the services of our legal adviser, Mr. Robert J. Callaghan of Philadelphia (a member of the firm of SCHNADER, HARRISON, SEGAL, AND LEWIS, which handled the dissolution proceedings for the A. S. T. M.). The bonds were received finally on January 12, 1954. Along with the $2016.00 of Series J bonds bought during 1952, and the $4000.00 of Series K bonds purchased during 1953, this brought the purchase price of our bond holdings to $12,528.00.

When the Council voted on May 18th to invest "all funds not needed for operating expenses", our bonds, except the $4000.00 in K bonds, were surrendered and netted $9,972.50. This sum, plus $1000.00 from the NEWS account and $5,027.50 from our Treasury made it possible to invest $16,000.00 in Series K bonds (2.76 per cent interest), which brought our total holdings in K bonds to $20,000.00. The interest only from this investment is ear-marked for reviews in the JOURNAL, the balance needed to be taken from the JOURNAL profit for the current year.

In addition to the $20,000.00 in K bonds, we have on hand Series J bonds costing at purchase $198.00. This represents the investment of the life membership fee of Dr. Charles A. Werner. The interest only of this investment may be used for operating expenses of the Society. It is suggested that the bonds be held to maturity when the interest can be deposited in the Treasury and the principle reinvested.

It will be noted above in the "Statement of Cash Receipts and Disbursements" (Exhibit

B) that the total receipts ($27,581.92) were in near balance with the total disbursements ($25,336.75). As per last year, this was made possible by a considerable profit on the JOURNAL ($2,693.55), and $1000.00 transferred from the NEWS account. It is also important to note that following the $20,000.00 investment of our surplus we completed the year with a bank balance of only $2,243.82. Of this amount, $2000.00 was appropriated in previous years for use by the Editor for extra pages in the JOURNAL, leaving an unappropriated surplus of only $243.82. Since we are committed in the future to invest funds to provide additional interest for use in financing reviews in the JOURNAL, again next year our unappropriated surplus should be quite modest.

Recommendations.—(1) that hereafter the annual financial reports, "approved by a certified public accountant", include the accounts of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS. Besides conforming more nearly to the directives set forth in Article IV of the By-Laws, this procedure provides a protection to the NEWS Editor, which was lacking in the previous arrangement, (2) that the date for closing the financial books be September 30, to allow ample time for preparation of this report, (3) that during the next year the Secretary-Treasurer be bonded for $15,000 (Article IV of the By-Laws), (4) that, if circumstances permit, the Secretary-Treasurer be authorized to invest funds in Series K bonds to provide additional interest for reviews in the JOURNAL, and (5) that the Secretary-Treasurer be authorized during the next year to spend monthly for secretarial assistance a sum not to exceed $200.00.

Respectfully submitted,
JOHN E. LARSH, JR.
Treasurer

The Treasurer's report was accepted as read. The recommendations were voted upon individually and all except number three were accepted without change. The fidelity bond of the Secretary-Treasurer shall be at the recommended sum of $15,000, provided the signature of another Officer(s) is required for redemption of our Government Bonds. If investigation reveals that another signature is not required, the sum of the fidelity bond shall be increased to $25,000. Further discussion led to favorable action on two additional proposals. It was agreed that the interest on our Government Bonds would be used not only for JOURNAL reviews, as agreed upon previously, but for covering the costs involved in publishing the abstracts of papers on the program, if the Editorial Board approves the suggestion of the Council that the abstracts be published (see above, Report of the Editor of the JOURNAL). It was also agreed that hereafter Auditors will not be appointed to examine the books of the Treasurer and the Editor of TROPICAL MEDICINE AND HYGIENE NEWS, since under the present arrangement all of the financial accounts of the Society must be examined and approved by a C. P. A.

Report of the Auditors: "The accounts of the Secretary-Treasurer of the ASTM & H and the Editor of TM and HN covering the period of October 31, 1953 to September 30, 1954 have been examined.

The report of the C. P. A., Alton G. Sadler, as of October 25, 1954 indicates the financial position of the Society and TM & HN to be that shown in the reports of the Secretary-Treasurer and the Editor respectively. The C. P. A. confirmed the cash in banks directly, and found the U. S. Savings bonds to be in the name of the Society.

By examination of the ledgers, bank accounts and bank statements through September 30, 1954, we find the accounts to be in order.

Respectfully written,
AIMEE WILCOX
QUENTIN M. GEIMAN

The report was accepted by the Council.

Reports of Committees

Bailey K. Ashford Award. Submitted by John F. Kessel

The Committee voted unanimously in favor of Dr. Joseph Greenberg as the recipient of this award.

Charles F. Craig Lectureship Committee. Submitted by Asa C. Chandler

The Chairman reported the unanimous choice of Dr. Albert B. Sabin to deliver the nineteenth annual Charles Franklin Craig Lecture.

* *Development and Needs.* Submitted by Albert B. Sabin for Paul F. Russell

1. *Committee Members.*—F. J. Brady—*ex officio*, L. E. Rozeboom, P. F. Russell (Chairman), A. B. Sabin, T. H. Weller, A. Wolman.
2. *Objective.*—To explore ways in which the ASTMH might properly use its prestige and means to develop an increased awareness of the importance of tropical medicine and hygiene to the USA, a greater emphasis on teaching and research, more clearly-defined objectives, and a more effective coordination of activities in this field.
3. *Committee Conferences.*—No meetings of the Committee were feasible but considerable correspondence was carried on between committee members.
4. *General Opinions.*—There was agreement among the Committee members that to a great and increasing extent the health of tropical countries is of importance to the USA because (i) the welfare of the world requires development of the tropics with, among other things, an organized and continued attack on the ill-health that reduces the productivity of the people, the extension of animal husbandry and agriculture, and the opening up of new lands for populations overflowing from crowded regions; (ii) the Armed Forces of the USA are and will be compelled to include tropical areas in future plans; (iii) various Federal agencies are already spending large sums and have considerable personnel in tropical areas; (iv) the USA is dependent on tropical areas for many basic and strategic supplies, such as cocoa, coffee, fibres, hardwoods, minerals, oils, spices, sugar and waxes; (v) it is in the interests of the USA to develop wider markets in tropical areas for its manufactured goods; (vi) rapid and extensive transport of people and goods between the tropics and the USA makes the exchange of parasites and vectors of disease a definite menace.

   The Committee members believe that current American activities in research, practice, and teaching in tropical medicine and hygiene are too limited in scope, are not well-planned or effectively co-ordinated, and do not have clear objectives.

   The Committee concluded that the ASTMH could have effective influence in the development of tropical medicine and hygiene but that it is essential that the ASTMH clarify its own collective thoughts and aims, and formulate specific objectives. This can best be done, the Committee suggests, by setting up and sponsoring a high-level Commission, whose terms of reference would include:

   (i) the gathering of specific data pertaining to the economic, social and hygienic impact of tropical diseases on the USA; and the current research, educational, and prophylactic activities of the USA as related to tropical medicine and hygiene needs;

   (ii) detailed analysis of the nature of any lacunae, if, as the Committee believes, such do exist; and

   (iii) the formulation of specific proposals as to the manner in which the USA can best exert its influence to meet the situation as disclosed.

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* Ad hoc Committee.

This high-level Commission should be made up of influential lay and scientific members, in active service in Government, Armed Forces, universities, medical missions, journalism, industry, and commerce. There should be competence within the Commission to consider not only medical and hygienic, but also veterinary, agricultural, demographic, cultural, social, and economic aspects of tropical diseases and their control or elimination.

It would seem to be essential that such a Commission have a full-time Chairman, with a suitable staff and with provision for necessary office and travel expenses, as well as for meetings of the Commission.

5. *Recommendations.*—The Committee suggests that the ASTMH should sponsor a high-level *Commission for the Study of American Activities in Tropical Medicine and Hygiene in Reference to Needs and Potentialities.*

The Committee recommends that the Council of the ASTMH take action to implement this suggestion.

The report was accepted as read. After a discussion of the recommendations, the Council voted in favor of the first recommendation, and, in keeping with the second recommendation, agreed that a new committee should be appointed to study the best way for setting up such a commission. This new committee would have the job of drawing up a memorandum describing in detail the purpose, objectives, and functions of this committee. This would be done after informal discussions with appropriate representatives of the Government, Armed Forces, universities, educational foundations, medical missions, journalism, industry, and commerce. The purpose of these discussions would be to determine as far as possible the special interests of these groups in the tropics so that they may be incorporated in the writing of directives for this commission. Furthermore, it would be the tasks of this new committee to explore different ways in which the permanent high-level commission could be financed, and to find appropriate candidates for the fulltime staff of this commission.

* *Education Committee.* Submitted by Herbert G. Johnstone

It was considered a worthy project by the members of the Education Committee to obtain information relative to the present status of the teaching of parasitology and tropical medicine in the medical schools of the United States. It was the belief of the Committee that a concerted effort should be made to survey the present situation in this country with regard to the teaching of these subjects, since apparently little attention had been paid to their status since the publication in 1946 of the report prepared during World War II by the Committee of the Association of American Medical Colleges under the chairmanship of Dr. Henry E. Meleney. Accordingly a questionnaire was prepared by the chairman to be submitted to the deans of the medical schools in this country as well as to key personnel interested in the teaching of parasitology and tropical medicine. The questionnaire was submitted to the other two members of the Committee and was approved by them. At this time a letter was received from Dr. Henry Meleney stating that the Parasitology and Tropical Medicine Study Section of the Research Grants division of the National Institutes of Health had requested its chairman, Dr. William W. Frye, to make a survey on teaching, training and research in parasitology and tropical medicine in the medical schools of the United States. A questionnaire was inclosed with the letter similar to the one which had been prepared by the Education Committee. It was obvious that it would be useless duplication to submit two questionnaires covering approximately the same material. Dr. Meleney

* *Ad hoc* Committee.

very kindly consented to include the report of his survey in the report of the Education Committee. His findings will therefore be made available to the membership of the American Society of Tropical Medicine and Hygiene.

With the appearance of Dr. Meleney's questionnaire it was apparent to the Chairman that some other project would have to be undertaken by the Education Committee for this present year. It was suggested by the Chairman to the other members of the Committee that it would be worthwhile to consider the opportunities which are presently available for young physicians in this country to find positions in tropical areas where clinical experience in tropical medicine could be obtained. Previous education committees in the Society at rare intervals have investigated the possibilities of placement of our young physicians in foreign posts, but in most instances these committees have been primarily concerned with research aspects of tropical medicine and have paid little attention to the availability of positions offering practical clinical experience. In those medical schools in this country where emphasis is given to tropical medicine and where staff members approach the subject with enthusiasm, interest is frequently created on the part of a student who often expresses a strong desire to go to the tropics when he has completed his internship and residency training programs. It has always proved extremely difficult to find a position for him. With this in mind an effort was made by the Committee to obtain information regarding clinical positions available to our young physicians in hospitals situated in the tropics.

Letters were sent to a large number of medical directors or administrators of commercial firms with subsidiary companies in the tropics as well as to staff members or administrators of certain medical institutions and agencies with interest in tropical areas. The letters requested information regarding the availability of positions for young clinicians. Replies were received from most of those to whom letters had been sent. Many of the writers stressed that it is not an easy task to find clinical positions in the tropics for our young physicians. Salaried positions are extremely limited. Since the majority of the persons to whom the letters were addressed were in a particularly favorable position to know of the present availability of clinical openings in the tropics, it will be of interest to note some of their pertinent reactions, suggestions and observations.

Several of the respondents felt that while there were many hospitals or institutions in tropical areas which would offer splendid opportunities for training, the availability of salaried positions to insure support of a young clinician and often for his family as well were almost nonexistent. It was noted by some of the writers that there are certain difficult barriers which confront a clinician who seeks training in clinical tropical medicine. A great many of the foreign countries demand that a practitioner be a citizen of the country. One of the correspondents pointed out that there are few hospitals existing in areas in the tropics where there will be an adequate number of English-speaking patients. He believes that if our men had a knowledge of one or more foreign languages, which unfortunately few have, most foreign institutions would welcome a young American clinician providing of course that there would be no financial burden on the institution. To overcome the language barriers it was suggested that an interested clinician should attempt to find a position in an institution doing specialized work for one or two years and, then, after he had acquired proficiency in the language of the country, do his clinical work in native hospitals. He would then be able to converse with the patients, orderlies and members of the staff. The medical officer of a British subsidiary of an American firm who had lived many years in India believes "that it is difficult for earnest, good young doctors to gain experience in tropical medicine and at the same time live a reasonable life in say India, Indonesia or Pakistan where the nationals of the countries are content with the wage and a mode of living which would be unacceptable to most of us and would in fact prove very difficult for us". This respondent however feels that a young clinician from this country would be warmly welcomed in any of these sections of the Far East if he could stand the abrupt change from one country to another and from one standard of living to another.

Several American firms which have subsidiary companies in tropical countries do not employ American doctors but depend solely on local physicians to provide medical care

for their employees. One large American oil company with holdings in the Near East employs in the neighborhood of 50 American doctors. Most of these doctors are considered general practitioners for the English-speaking population and, unless they are particularly interested, may never come in contact with medical problems peculiar to the natives of the region. In this connection one of the correspondents believed the medical man trained in most of the schools in the United States would profit a great deal by the chance to see the severe types of tuberculosis, pneumonia and enteric infections which are still commonplace in most tropical countries.

It is evident from the information supplied by the numerous respondents that the opportunities presently available for young graduates of medical schools in the United States to gain clinical experience in tropical medicine on a salary basis are extremely limited. In fact in all of the correspondence received so far there was not one instance cited where a salaried position was available which would insure a good basic training in clinical tropical medicine.

It is apparent from the evidence so far received that those young clinicians who are desirous of working in tropical hospitals would find it impossible to locate a position which would provide an income for themselves as well as their families. In all probability if opportunities for training are to be made available to these men it will be necessary to find a source of income for them during their training period abroad. Some type of fellowship, grant-in-aid or other means of support from a government or private agency will be necessary. It may be that the suggestion made by Dr. Henry Meleney to the Chairman would at least in part help considerably in solving the problem. Dr. Meleney believes that a concerted effort should be made to establish a center somewhere in the tropics where interested persons could obtain adequate training in tropical medicine. Dr. Meleney suggests that funds should be sought to establish such a center and appropriated to one of the medical schools in this country which would supervise the program. In planning such a program Dr. Meleney believes sufficient funds should be appropriated to pay the hospital and the director of the hospital for their contribution to have an American physician as director of the program on a full-time or part-time basis and to have funds for fellowships which would be awarded to qualified applicants, both graduate and undergraduate. It may be that a suitable place in the tropics could be found among those noted in the following list: 1) Hospital San Juan de Dios—San José, Costa Rica; 2) Institute of Tropical Medicine—Mexico City; 3) University of Puerto Rico; 4) School of Medicine, University of Mexico; 5) School of Medicine, American University of Beirut; 6) Venezuelan Institute of Malariology at Maracay; 7) United States Navy Medical Research Facility in Cairo, Egypt (NAMRU-3); 8) Two hospitals in Peru maintained by R. W. Grace and Company, located at Paramonga and at Cartavio; 9) Medical Service and Hospital of the Arabian American Oil Company in Saudi Arabia; 10) The University College of the West Indies, Mono, St. Andrew, Jamaica, B. W. I.; 11) Hospital de Enfermedades de la Nutrición—Mexico City; 12) Hospital Infantile—Mexico City; 13) Medical School in Cuba; 14) School of Medicine, University of Manila; 15) The University of Malaya at Singapore; 16) The Medical School of Makerere College at Kampala, Uganda, Africa.

Honorary Membership. Submitted by George R. Callender

No nominations were received during the past year.

Joseph Augustin LePrince Award. Submitted by Frederick J. Brady

The Chairman reported the unanimous choice of Dr. Brian G. Maegraith, Dean of the Liverpool School of Tropical Medicine, to receive this Award.

Membership Committee. Submitted by J. Clyde Swartzwelder

The Committee has reviewed and approved the applications for membership of 58 individuals. Recently, invitations to apply for membership in the Society were sent to 82 mem-

bers of the professional staff of the Communicable Disease Center, Public Health Service, Atlanta and Chamblee, Georgia. Some response to this endeavor is already being received. There are many other persons associated directly or indirectly with the CDC in the various states, who may be interested in affiliating with the Society. Future committees on membership may wish to consider contacting these individuals.

The data on membership, provided by the Secretary of the Society, are as follows: Honorary Members—17, Emeritus—6, Life Member—1, Active Members in good standing—1,070, Active Members in arrears for one or two years—87; total membership, 1,181.

It is suggested that consideration be given to the possibility of printing a new brochure on the nature and objectives of the Society. This may be useful for future membership campaigns or individual solicitation.

* *Professional Relations.* Submitted by Hamilton H. Anderson

Your committee on *Professional Relations* was charged specifically with concerning itself with the American Medical Association and subsidiary bodies for the promotion of Tropical Medicine. Accordingly a committee was set up comprised of the following members: Dr. E. H. Lennette, Berkeley; Dr. G. M. Saunders, N. Y. C.; Dr. E. H. Hinman, Puerto Rico; Dr. Q. M. Geiman, Boston.

Each member of the committee was asked to submit ideas as to procedure and possible objectives. At the outset, it was believed desirable to have an open letter sent to the Editor of the J. A. M. A., Dr. Austin Smith, signed by the Presidents of the Society and of the Academy.

This was done and the J. A. M. A. was encouraged to continue to publish lead articles such as appeared in November, 1953, on "Increasing Incidence of Bacillary Dysentery" by Felsen and Wolarsky; and "Responsibilities of the Physician in World Affairs" by J. R. Trimble. Thus, it became our objective to have physicians practicing in the United States develop an "index of suspicion" toward Tropical Diseases which we believe to be important for the health of Americans wherever they may be. It would appear from an analysis in a recent issue of the U. S. News and World Report that there are far more countries, outside the U. S. A., to which Americans are assigned at present than during World War II.

A second step was taken in meeting with Dr. Dwight Murray, Chairman of the Board of Trustees of the American Medical Association, and his support was elicited to further the objectives of our Society by rekindling interest in tropical medicine inside the U. S. A. Following these initial meetings with Dr. Murray, whose sympathy was readily gained, your President, Dr. Frederick Brady, met with a new A. M. A. Committee on International Health Activities. A report will probably be forthcoming on the results of these meetings.

Finally, a third activity of members of your committee and others in the Society was participation in the June, 1954, meeting of the A. M. A. Dr. Brady met with Doctors Diehl and Hanlon to present a resumé of the activities of the World Health Assembly, particularly those aspects dealing with the United States. The potency of health activities of our governmental and non-governmental agencies abroad is not fully appreciated, as it should be, by either the medical profession in this country or the Department of State. Some emphasis on this aspect of the subject as it relates to Tropical Medicine deserves further study.

In San Francisco, June, 1954, a half-day session of the Section on Preventive Medicine was devoted to International Health problems, in which members of the Society, notably Doctors Brady, Soper, Hackett, and Anderson participated. International educational problems, changing incidence of tropical and sub-tropical diseases, F. O. A. programs, and the role of the physician in international health were stressed in this symposium, which will be published in the J. A. M. A.

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* *Ad hoc* Committee.

The report of this Committee was accepted as read, and the Council agreed that this Committee should be continued.

Program Committee. Submitted by John E. Larsh, Jr.

In mid-February, the Committee was requested to consider items concerned with overall plans for the Memphis meeting. There was general agreement on the following: (1) that the meeting this year should open on Wednesday afternoon and adjourn Saturday noon, (2) that we should hold 2 meetings of the Council (one Wednesday, November 3, at 9:00 A.M., the other that evening), (3) that we should agree to sponsor with the American Society of Parasitologists a symposium and omit a meeting of our group during the afternoon of their demonstrations, and (4) that the topic "Schistosomiasis" with Dr. McMullen as Moderator should be given careful consideration. On March 5, the Officers and Councilors voted favorably upon the recommendations of the Committee and specific plans for the meeting were begun. Later in March, the Committee approved a tentative program summary. On April 10, all members of the Society were sent an announcement of the meeting and were requested to submit before July 1 applications and abstracts for papers and/or scientific exhibits. In all, there were received 62 requests to read a paper and 6 requests to mount a scientific exhibit. In mid-July, the applications and abstracts were sent to the members of the Committee for review and action. It was decided to accept all of the requests to mount a scientific exhibit. Of the 62 requests to present a paper, 50 were accepted and the remaining 12 were placed in the group "to be read if time permits." To accommodate the largest possible number of papers, it was decided to follow last year's decision to limit all papers to 10 minutes.

* *Society Organization.* Submitted by E. Harold Hinman

Part A.—Your Committee has proposed the following changes in the Constitution, and following approval by the Council the matter has been circulated for vote of the entire membership.

Constitution—Article II

(a) Section 2: "There shall be *five* classes of members: Active, Honorary, Life, Emeritus, and *Fellow*."

(b) Section 7: (*new*), "Active members of the Society who have had not less than five years of continuous membership and who have made meritorious contributions to tropical medicine and/or hygiene may be elected to Fellowship as provided for in the By-Laws of the Society. Fellows shall pay the same dues as active members and shall continue as Fellows so long as their membership is in good standing."

The Committee proposed, and the Council approved, the following changes in the By-Laws. These will be voted upon by the members present at the business meeting Saturday, November 6.

By-Laws

(a) Article X (*new*) "Fellows shall be elected by a two-thirds majority vote of the officers and councilors of the Society."

(b) Present articles X–XIII to be elevated by one number to bring the total to XIV.

Part B.—Last year a committee recommended to the Council of the American Society of Tropical Medicine and Hygiene the establishment of a permanent secretarial office for the Society.

Your committee has considered the implications of this recommendation and has reached the following conclusion. We are heartily in favor of giving all the necessary secretarial help to our Secretary-Treasurer so that the work may be carried on efficiently and so long as he is able to obtain satisfactory office assistance we believe the Society could employ

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* *Ad hoc* Committee.

to better advantage the funds that would be required to rent separate office space and set up such a non-professional office. We recommend that the Society should continue to study this problem and that if at some future date the Editor's office, the office of the Editor of the Tropical Medicine and Hygiene News, and the Secretary-Treasurer's office should be located in the same city, that there would be some advantage to the establishment of a permanent secretarial office for the Society. Since it is apparent to the committee that the incumbent Secretary-Treasurer feels that he is obtaining adequate assistance, we would be most reluctant to recommend any change at this time.

The report was accepted as presented including the recommendations listed in part B.

All of the above reports were accepted by the Council with appreciation for the work of the various committee members.

Reports of Representatives to Organizations

A.A.A.S. Submitted by Quentin M. Geiman

The two Council meetings of the AAAS held on December 27 and 29, 1953, in Boston, were attended and the following brief report is made.

The major part of the agenda of the two meetings concerned the routine affairs of the Society, the election of new officers, and the reports of standing committees, including the Publication Committee and the Building Committee. The roster of newly elected officers has been published in SCIENCE and the reorganization of the Editorial Staff of publications was announced and has since been put into operation.

A major item under new business concerned a resolution about the approval of President Eisenhower's plan given to the United Nations concerning the stock-piling of atomic energy. Although there was some opposition to approval of a resolution with political implications, it was felt and so voted by the Council members present that the AAAS should take a stand in a matter of this type regardless of the political implication.

The retiring president, Dr. Detlev W. Bronk, presented his annual report, including a review of administration difficulties and pertinent statements about the publications of the organization, SCIENCE and THE SCIENTIFIC MONTHLY. Efforts were to be made by the new Editorial Staff and the two additional members of the Editorial Board to improve the publications of the organization, increase the amount of advertising and to explore ways of increasing revenue. An Advisory Committee was appointed to consider the publication of a new edition of AMERICAN MEN OF SCIENCE. During the past year there was an increase of 12 associated societies with a slight gain in membership.

Mr. Bentley Glass, chairman of the Editorial Board, requested that Council members during the coming year call outstanding events in their own field to the attention of the Editorial Board. Better coverage of scientific events is being attempted, but help from outside the Editorial Board is needed to accomplish this end.

Registration at the Boston meeting included approximately 3,000 persons. Meeting places for the AAAS in the future are San Francisco in 1954, Atlanta, Georgia in 1955, New York City in 1956, Indianapolis in 1957, and Washington, D. C. in 1958.

The financial position of the Society appears to be strong, with approximately $100,000 being added to the funds during the past year. During the coming year a study is to be made of the business operations of the AAAS, its budgetary procedures and its publication policies.

In closing this report, the AAAS is a national organization made up of scientists and professional men representing many fields of knowledge, but it is passing through a transition in the type of annual meetings that it holds. Membership has become so large and affiliated organizations so numerous that the planning of scientific programs and symposia is becoming increasingly difficult. A large number of organizations, although still affiliated

with the AAAS, are holding their meetings alone or with a few closely related organizations at another time of year.

Even though the ASTMH might not wish to schedule its annual meeting along with that of the AAAS, the AAAS deserves its support. SCIENCE should be routinely notified about Society affairs of national and international importance, elections, and special events in order to build up closer liaison with the AAAS. The AAAS represents one national organization that can speak for scientists to the laymen and represent the fields of science collectively to similar organizations in other countries. Therefore, continued appointment of members to the Council of the AAAS should be practiced so that our organization can be informed about the transactions of this most important organization.

American Foundation for Tropical Medicine. Submitted by Edward I. Salisbury

Up to October 1, 1954 the income of the Foundation has been substantially the same as for 1953. Because of the urgency of the Liberian Institute, it has been necessary to concentrate the financial efforts of the Foundation on the operation of the Institute in Liberia. There have been no grants-in-aid for tropical medicine with the exception of that to "Tropical Medicine and Hygiene News". The Foundation still pays one-third of the cost of the printing and mailing which amounts to approximately $650.00 per year.

Liberian Institute.—Dr. Max Miller is still Director in Charge. In October of this year, we sent Dr. Richard M. Fox to Liberia as Medical Entomologist. He was accompanied by his wife who is also an Entomologist and Zoologist and will probably take an active part in the laboratory work at a later date. The employment of a Parasitologist with a medical degree is now pending. During the year a community house was completed. This building will house the mess and have rooms for single workers. Our other living quarters consist of three houses for married couples. During the year Dr. Miller was engaged in therapeutic trials of several drugs in malaria, trypanosomiasis, schistosomiasis, and also on the effects of milk diet on the resistance to malaria. The laboratory is also engaged in the basic work of disease incidence and disease vectors. The laboratory is available for use of individuals or universities that wish to carry out work in some branch of tropical medicine. Laboratory space is available as well as living quarters for single personnel.

The number of industries interested in Africa is limited but the field of tropical research should be of interest to organizations working in other parts of the world, and we are inviting the members of the American Society of Tropical Medicine and Hygiene to assist us in getting financial support for the Foundation.

National Research Council. Submitted by Harry Most

The Annual Report of the Division of Medical Sciences, National Research Council, was held in Washington at the National Academy of Sciences on May 22, 1954. The President of the National Academy of Sciences, Dr. Detlev W. Bronk, and the Chairman of the National Research Council, Dr. William W. Rubey, addressed the members of the Division of Medical Sciences Executive Committee, the Representatives of Societies and the Government, Liaison Representatives and guests in attendance at the Annual Meeting. Dr. R. Keith Cannon, Chairman of the Division, outlined the various administrative functions and activities of the Division and emphasized the magnitude, scope and diversity of its operations. Fellowships, program and policy guidance were discussed and representatives heard from the Committee on Veterans Medical Problems and General Advisory Service. The latter included Dental Research, the National Blood Program, Infectious Diseases, Psychiatry and Sanitary Engineering. Special Activities reported on included Atomic Bomb Casualty Commission, Cancer Diagnosis and Therapy and Pathology. The relationships of the Division with government agencies and its cooperating societies were discussed and finally reports of the cooperating societies were presented.

It is of interest that at this meeting there was no report from the Panel on Malaria. A brief report of the Panel on Malaria which is a standby Panel on Subcommittee on Infectious Diseases and Chemotherapy is contained in the Annual Report of the Division of

Medical Sciences of the National Research Council. In this report it is stated that the membership in the Panel on Malaria had been reduced because of the decrease in importance of the problem of malaria in the armed forces. However the Panel is monitoring Panel studies on volunteers to determine whether chemosuppression with chloroquine can be strengthened with the addition of primaquine. The Panel is planning to bring the history of the activities of its predecessor advisory group in the post-war period up to date.

A new development in the activities of the Division of Medical Sciences is a meeting scheduled for November 1954 in addition to the annual meeting in May to explore among other things more intimate relations between the division and the participating societies.

These various reports were accepted as read.

Report of the Custodian of Back Issues, the JOURNAL *of the NMS.* Submitted by G. Robert Coatney

The following shipments have been made: 51 in the U. S., 11 to foreign countries, 31 to the Pan-American Sanitary Bureau (including 10 symposium copies), and 2 to FOA (including 22 sets and 95 symposium copies). The following is a list of the remaining issues: 2 sets of volumes V–X, approximately 55 sets of volumes VI–X, approximately 250 copies of the symposium number, and miscellaneous numbers.

This report was accepted as read. In the discussion that followed, suggestions were presented for disposing of the remaining copies. So that our members would have first priority, it was decided to announce at the business meeting on November 6 that certain copies are available, the only charge being for postage. It was also suggested that the next priority should be libraries, here and abroad, and that they should be made aware of this offer by an announcement in SCIENCE and/or other publications, or by other appropriate means.

Election of Officers and Councilors for 1955

The Nominating Committee (Paul F. Russell, Chairman; Lawrence B. Hall; and Herbert G. Johnstone) submitted a slate which was mailed with a ballot to all voting members of the Society. The votes were tabulated by the Tellers, Thomas A. Burch and John B. Poole, appointed by the President. The results of the election were as follows:

President-Elect	ASA C. CHANDLER
Vice-President	DONALD B. MCMULLEN
Councilors	MARSHALL C. BALFOUR
	THOMAS H. WELLER

Other Old Business

The only remaining item of old business concerned the medal commemorating the fiftieth anniversary of the founding of the A.S.T.M. Since the entire order of 100 medals placed on sale at the Louisville meeting has been sold, and since 25 additional orders have been received, it was voted to place a new order for 50 medals.

New Business

Committee Appointments.—In addition to the appointment of Auditors and Tellers for this meeting (see above), the President appointed Hamilton H. Ander-

son as Chairman and Ernest Carroll Faust and Thomas H. Weller as other members of the Resolutions Committee.

The Council elected the following members to serve on the Nominating Committee for 1955: Thomas F. Whayne, Chairman; John M. Henderson; and Leon H. Schmidt.

Contribution to the National Society for Medical Research.—After discussion of the program of this organization, a contribution of $25.00 was voted.

Consideration of Affiliation with the American Institute of Biological Sciences.—A recommendation that the Society affiliate with the A.I.B.S. was given careful consideration. When the discussion led to general agreement that, considering the composition of our membership and other pertinent factors, affiliation would not seem to be indicated, a motion was entered that we not join this group. This motion was given favorable action.

Invitations to Members of Other Societies.—It was the feeling of the group that it would not be desirable to take steps calculated to increase greatly the size or to increase the range of interests of our membership. Therefore, it was decided not to invite as a group the members of another organization to file applications for membership, as had been suggested earlier.

Invitation from the International Science Foundation.—Following a discussion concerning this organization, its history, objectives, etc., it was voted to decline the invitation of the Board of Governors to our Society to become a participating member. It was agreed in general that we should give further consideration to this offer at a later time.

1955 Meeting.—More than a year ago, the city of Boston through its Chamber of Commerce extended an invitation to meet there in 1955. This invitation was supported by many groups, including the School of Public Health of Harvard University. In view of the many points favoring Boston as a meeting site for our group, the Council, at the Louisville meeting, suggested that the Secretary-Treasurer give careful consideration to this invitation. In April of this year, the Secretary-Treasurer visited Boston to inspect personally the facilities available and to discuss details with certain local members of the Society. The facilities proved to be quite adequate and there was assurance that several members would serve on a Local Committee. Thus, the Secretary-Treasurer recommended, and the Council approved, Boston as the 1955 meeting site, and the dates of November 2–5 were approved.

Ad Hoc Committees for 1955.—The Council authorized the President to appoint such committees that he and the Secretary-Treasurer deem necessary.

Procedures Initiated at the Memphis Meetings.—Since it was agreed that the bound abstracts of papers on the scientific program and the list of registrants at the meetings served useful purposes, it was suggested that these procedures be continued.

MINUTES OF THE ANNUAL BUSINESS MEETING

The annual business meeting of the Society was held November 6, 1954 at 8:30 A.M. in the Ballroom of the Hotel Peabody, Memphis, Tennessee, with President Frederick J. Brady presiding.

The minutes of the second meeting (Louisville, Kentucky, November 14, 1953) were accepted as published in the JOURNAL, May, 1954.

The Secretary-Treasurer's reports were approved as read.

The Editor-in-Chief of Publications, Lewis W. Hackett, presented the JOURNAL report, which was accepted as read. In the discussion that followed, the members were requested to support the firms using our JOURNAL as an advertising medium and to mention the JOURNAL when discussing orders, etc. with their agents.

The Editor of TROPICAL MEDICINE AND HYGIENE NEWS, Leon Jacobs, read his report, which was accepted.

The report of the Auditors, Quentin M. Geiman and Aimee Wilcox, was read by the Secretary-Treasurer. This report pointed out that the accounts of the Treasurer and of the Editor of TROPICAL MEDICINE AND HYGIENE NEWS were found to be in order.

Reports of committees and representatives.—The various reports presented at the Council meeting (see above) were summarized. Approval of these reports was voted.

Announcement of the report of the Custodian of back issues, the Journal of the NMS.—The data in the report given at the Council meeting by G. Robert Coatney were presented, and the members were reminded of the availability of certain copies.

Survey of the teaching of parasitology and tropical medicine in the medical schools of the U. S.—Henry E. Meleney gave a 15-minute report on the findings of this survey. Details are being omitted here, since plans call for publication of the survey in full in a forthcoming issue of the JOURNAL.

Announcements.—The results of the election of Officers and Councilors by mail ballot were given. Asa C. Chandler was elected President-Elect of the Society for 1954–55, Donald B. McMullen was elected Vice-President, and Marshall C. Balfour and Thomas H. Weller were elected Councilors for a term of four years.

It was announced that the Council had voted a $25.00 contribution to be sent to the National Society for Medical Research, and that the Council had appointed the following members to serve on the 1955 Nominating Committee: Thomas F. Whayne, Chairman; John M. Henderson; and Leon H. Schmidt. It was also announced that the Council had authorized the President to appoint *ad hoc* committees for next year, and that it had been decided to order, for sale to the members and other interested parties, 50 additional medals commemorating the fiftieth anniversary of the founding of the American Society of Tropical Medicine. Finally, it was announced that the Council had accepted the invitation of Harvard University and the Boston Chamber of Commerce to hold the 1955 annual meeting in Boston. The meeting will be held November 2–5, 1955.

New Business.—It was announced that as a result of the mail ballot, sent in September, the Constitutional amendment to provide for FELLOWS in the Society had been adopted (of the ballots received, 335 voted in favor of the amendment and only 28 voted to the contrary). This opened the way for consideration of the following amendment to the By-Laws covering the procedure for election of FELLOWS: Article X (*new*) "Fellows shall be elected by a two-third majority

vote of the Officers and Councilors of the Society" (Present Articles X–XIII to be elevated by one number to bring the total to XIV). After presenting these proposed changes, a vote was taken (the Tellers, Thomas A. Burch and John B. Poole counted the votes taken by a show of hands). There was only one dissenting vote among the 31 members who voted, so that the condition for adoption of the changes was met (Article XIII of the By-Laws: "The By-Laws may be amended at the annual meeting by a two-third vote of members present and voting . . . ."). Following adoption of these changes in the By-Laws, a motion to elect to fellowship all eligible members of the recently dissolved American Academy of Tropical Medicine was defeated.

A favorable vote was received on a motion that the Society accept from the dissolved Academy its archives, seal, and remaining financial assets.

Report of the Resolutions Committee (Hamilton H. Anderson, Chairman; Ernest Carroll Faust; and Thomas H. Weller):

I. It is requested that the Secretary of the Society be instructed to write and send resolutions of sympathy to the families of deceased Active and Honorary Members of the American Society of Tropical Medicine and Hygiene. During the current year, the Society has lost three pioneers and outstanding leaders, namely, Virgil Cornell, James Stevens Simmons and S. Burt Wolbach.

II. The Officers and Members of the American Society of Tropical Medicine and Hygiene have benefited by the symposium arranged for this meeting. THEREFORE BE IT RESOLVED that the Society extend congratulations and appreciation to the organizers and participants in the symposium on "Schistosomiasis", moderated by Dr. Donald B. McMullen.

III. To Dr. Albert B. Sabin, we extend this Society's sincere thanks and highest esteem for his able presentation of the 19th Annual Charles Franklin Craig Lecture, "*Recent advances in our knowledge of dengue and sandfly fever*".

IV. To the organizations and individuals who have served as hosts for the Third Annual Meeting of the Society, notably the University of Tennessee, the Memphis-Shelby County Medical Society, Memphis Academy of Internal Medicine, and the Laboratory of Tropical Diseases, National Institutes of Health, Memphis.

A. The Officers and Members of the American Society of Tropical Medicine and Hygiene are deeply appreciative of the assistance and hospitality extended to the Society on the occasion of the Third Annual Meeting held in Memphis, Tennessee, November 3–6, 1954, by the University of Tennessee. THEREFORE BE IT RESOLVED that the thanks of the Society be expressed to Dr. O. W. Hyman, Dean and Vice President, and through him to the University of Tennessee.

B. The Memphis Chamber of Commerce has contributed greatly to the success of the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Officers and Members be extended to Mrs. Elizabeth Crane, Secretary of the Bureau of Conventions of the Memphis Chamber of Commerce.

C. The assistance and cooperation of the members of the staff of the Peabody Hotel contributed to the success of the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954. THEREFORE BE IT RESOLVED that the thanks of the Officers and Members of the Society be extended to Mr. T. J. McGinn, Vice-President and Assistant Manager, and through him to the other members of the staff of the Peabody Hotel.

D. The foresight and planning of the Local Committee on Arrangements, were essential contributions to the success of the Third Annual Meeting of the American Society of Tropi-

cal Medicine and Hygiene, November 3–6, 1954. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Officers and Members be expressed to the Chairman of the Local Committee, Dr. Don E. Eyles and Committee members as follows: Miss Frances E. Jones, Miss Nell Coleman, Dr. Colvin L. Gibson, Dr. Frank L. Roberts, Mr. Fred L. Knowles, Dr. Wheelan D. Sutliff and Dr. Arne V. Hunninen, and through them to the other members of the host organizations who have assisted with the arrangements for the meeting.

E. The interest and planning of the Committee for Ladies' Entertainment contributed greatly to the pleasure of the ladies attending the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954. THEREFORE BE IT RESOLVED that the Officers and Members of the Society express their thanks and appreciation to the Co-Chairman, Miss Frances E. Jones.

F. The arrangements of the Committee for Ladies Entertainment sponsored by Kay Surgical Company added greatly to the pleasure of the ladies attending the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Officers and Members of the Society be extended to Mr. Harvey L. Kay of Memphis.

G. The Hospitality Sessions held at the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954, were a most important contribution to the pleasure of the individual members and guests and to the success of the meeting as a whole. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Officers and Members of the Society be extended to:

(1) Dr. Edward I. Salisbury and through him to the United Fruit Co., host for the Hospitality Session on Wednesday, November 3rd.

(2) To Mr. Howard Fonda and through him to Burroughs Wellcome and Co., host for the Hospitality Session on Thursday, November 4th.

(3) To Dr. Justus B. Rice and through him to Winthrop-Stearns, Inc., host for the Hospitality Session on Friday, November 5th.

H. The Commercial Exhibits at the Third Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 3–6, 1954, added materially to the interest of the meeting and assisted the Society in defraying the costs. THEREFORE BE IT RESOLVED that the thanks and appreciation of the Officers and Members of the Society be expressed to Mrs. Herma H. Hoefler, of the American Foundation for Tropical Medicine, through whose efforts the exhibits were obtained.

V. *Editors of Society Publications*

A. Dr. Lewis W. Hackett, Editor of the American Journal of Tropical Medicine and Hygiene is to be commended for his efforts which have resulted in a Journal of outstanding quality. THEREFORE BE IT RESOLVED that an expression of thanks on behalf of the Society be extended to him.

B. Dr. Leon Jacobs is to be commended for editing the timely and interesting Tropical Medicine and Hygiene News. THEREFORE BE IT RESOLVED that an expression of appreciation of the Society be extended to him.

VI. *Other Resolutions*

Since epidemic yellow fever arising in Panama in 1948 has progressed steadily northward and has appeared recently in Honduras threatening Caribbean and Gulf ports wherever *Aedes aegypti* occurs, and since the appearance of yellow fever in the Island of Trinidad in 1954 led to the imposition of international quarantine with disastrous economic consequences, and the arrival of a single case of yellow fever in an infested port, even though danger of spread could be minimized by emergency measures, would undoubtedly provoke national and international reactions with serious social and economic consequences,

THEREFORE The American Society of Tropical Medicine and Hygiene takes note of the potential seriousness of this development and urges that appropriate action should be taken to reduce, and, where possible, eradicate *Aedes aegypti* from infested ports in the U. S. A.

Since one of the members of the Society has been distinguished by being awarded the

Nobel Prize in Medicine for participation in fundamental studies relative to poliomyelitis, the American Society of Tropical Medicine and Hygiene wishes to congratulate Dr. Thomas Weller for this signal honor.

This report was approved as read, and a motion for adjournment was passed at 9:50 A.M.

Respectfully submitted for publication,
JOHN E. LARSH, JR.
Secretary-Treasurer

SCIENTIFIC SESSIONS

A total of five half-day sessions was held, four for the presentation of papers and awards, and one for a symposium sponsored jointly with the American Society of Parasitologists. All sessions were held in the Ballroom of the Hotel Peabody.

The first scientific session was held on Wednesday afternoon, November 3, 1954. President Frederick J. Brady presided.

2:00 P.M., November 3

Presentation of the Bailey K. Ashford Award (established by Eli Lilly and Company) by JOHN F. KESSEL, Chairman of the Award Committee.

1. *The Effectiveness of Long and Short-Term Suppressive Chloroquine-Primaquine Treatment for the Eradication of* Plasmodium vivax *Infection.* G. ROBERT COATNEY, CLARENCE A. IMBODEN, JR., and HERBERT BURTON, National Institutes of Health, Bethesda, Md.
2. *Single Dose Treatment With Chloroquine Against Clinical Attacks of Chesson Strain* vivax *Malaria.* CLARENCE A. IMBODEN, JR., W. CLARK COOPER, and G. ROBERT COATNEY, National Institutes of Health, Bethesda, Md.
3. *The Hemolytic Effect of Primaquine.* RAYMOND J. DERN, ERNEST BEUTLER, and ALF S. ALVING, Stritch School of Medicine, Loyola University, Chicago, Ill. and Army Malaria Research Unit of the Department of Medicine, University of Chicago, Chicago, Ill.
4. *A Study of Primaquine-Sensitive Erythrocytes.* ERNEST BEUTLER, RAYMOND J. DERN, and ALF S. ALVING, Army Malaria Research Unit of the Department of Medicine, University of Chicago, Chicago, Ill. and Stritch School of Medicine, Loyola University, Chicago, Ill.
5. *Mitosis in* Plasmodium berghei. EDWARD D. DELAMATER, School of Medicine, University of Pennsylvania, Philadelphia, Pa.
6. *Effect of Chemotherapeutic Agents on the Nuclear Apparatus* of Plasmodium berghei. *I. A. Comparison of Pyramethamine (Daraprim) and Chloroquin (Aralen).* EDWARD D. DELAMATER and RALPH JONES, School of Medicine, University of Pennsylvania, Philadelphia, Pa.
7. *Description of the Developmental Stages of Avian and Simian Malaria.* HARALD N. JOHNSON, Division of Medicine and Public Health, The Rockefeller Foundation, New York, N. Y.
8. *Experimental Testing of the Susceptibility of the Negro to* vivax *Malaria.* MARTIN D. YOUNG, DON E. EYLES, ROBERT W. BURGESS, and GEOFFREY M. JEFFERY, National Institutes of Health, Columbia, S. C., Memphis, Tenn., and Milledgeville, Ga.
9. *A Comparison of West African and West Pacific Strains of* Plasmodium ovale. GEOFFREY M. JEFFERY, AIMEE WILCOX, and MARTIN D. YOUNG, National Institutes of Health, Milledgeville, Ga. and Columbia, S. C.
10. *Preservation of Viable Human Malaria Sporozoites by Low Temperature Freezing.* GEOFFREY M. JEFFERY and

ROBERT C. RENDTORFF, National Institutes of Health, Milledgeville, Ga. and Columbia, S. C.

Presentation of the Joseph Augustin LePrince Award (honorarium provided by the Michigan Chemical Corporation) by FREDERICK J. BRADY, Chairman of the Award Committee.

11. *Estimation of Population and Birth and Death Rates of the House Fly* (Musca domestica). FREDERICK L. KNOWLES and H. AKINS (Introduced by DON E. EYLES), National Institutes of Health, Memphis, Tenn.
12. *Infrared Spectrometry of the* Culex pipiens *Complex*. DON W. MICKS, Medical Branch, The University of Texas, Galveston, Tex.
13. *Water Management Planning for Malaria Prevention in the Damodar Valley, India*. JOHN M. HENDERSON, Communicable Disease Center, Savannah, Ga.
14. *The Effect of Chemical Treatment Upon the Types of Flies Produced in Privies*. JOHN W. KILPATRICK and HERBERT F. SCHOOF, Communicable Disease Center, Savannah, Ga.

The second and third scientific sessions were held during the second day of the meeting, Thursday, November 4, 1954. The third, a morning session for the presentation of papers, was presided over by President Frederick J. Brady. The fourth, an afternoon session devoted to a symposium sponsored jointly with the American Society of Parasitologists, was presided over by the Vice-President of the ASP, Donald B. McMullen, who also served as Moderator.

9:00 A.M., November 4

15. *Studies of a Water-Borne Outbreak of Amebiasis in South Bend, Indiana*. III. *Prevalence Rates in Family Contacts*. MARION M. BROOKE, DOROTHY M. MELVIN, ROBERT SAPPENFIELD, FRED PAYNE, W. W. FRYE, F. R. CARTER, and A. C. OFFUTT, Communicable Disease Center, Atlanta, Ga., School of Medicine, Louisiana State University, New Orleans, La., Department of Public Health, South Bend, Ind., State Board of Health, Indianapolis, Ind.
16. *Experiments on the Growth of* Endamoeba histolytica *in Dialysate and Heat-Treated Media*. CHARLES W. REES and LUCY V. REARDON, National Institutes of Health, Bethesda, Md.
17. *Experimental Studies in Establishing Multiple Intestinal Amebic Infections in Man*. ROBERT C. RENDTORFF, National Institutes of Health, Columbia, S. C.
18. *The Use of Penicillin and Streptomycin in the Routine Cultivation of Amebae from Fecal Specimens*. LOIS NORMAN and MARION M. BROOKE, Communicable Disease Center, Atlanta, Ga.
19. *A Study on the Pathogenicity of Various Strains of* Endamoeba histolytica. ARNE V. HUNNINEN and HOWARD A. BOONE, School of Medicine, University of Tennessee, Memphis, Tenn.
20. *Erythromycin Stearate against Systemic Amebiasis in Colombia*. HAMILTON H. ANDERSON, THOMAS L. NELSON, C. CARBONO, and JORGE CAMACHO, School of Medicine, University of California, San Francisco, Calif., and United Fruit Co., Santa Maria, Colombia.
21. *Observations on the Effect of Oral and Intravenous Aureomycin and Terramycin in the Treatment of 17 Patients with Amebic Liver Abscesses*. ELVIO H. SADUN, VIKIT VIRANUVATTI, and TRANAKCHIT HARINASUTA, Communicable Disease Center, Atlanta, Ga. and Siriraj University Hospital, Bangkok, Thailand.
22. *Laboratory Studies on the Antiamebic Action of Camoform*. PAUL E. THOMPSON, JAMES W. REINERTSON, DUNCAN A. MCCARTHY, and ANITA BAYLES, Parke, Davis and Company, Detroit, Mich.
23. *Milibis as a Prophylactic Against Experimental* Endamoeba histolytica *Infection in Man*. PAUL C. BEAVER,

RODNEY C. JUNG, and THOMAS R. READ, Tulane University School of Medicine, New Orleans, La.

24. *The Effect of Rabbit Serum on the Ability of* Endamoeba histolytica to *Phagocytose RBC.* JAMES G. SHAFFER and JOSEPH ANSFIELD, The Chicago Medical School, Chicago, Ill.
25. *The Utilization of Glucose-C^{14} by Two Strains of* Entamoeba histolytica. CHARLES E. BECKER and QUENTIN M. GEIMAN, School of Public Health, Harvard University, Boston, Mass.
26. *Motility of Parasitic Amoebae in Vitro.* MALCOLM S. FERGUSON and LOIS NORMAN, Communicable Disease Center, Atlanta, Ga.
27. *Variations in Occurrence of* Endamoeba histolytica *in Multiple-Stool Examinations.* LYMAN P. FRICK, DALE E. WYKOFF, and LAWRENCE S. RITCHIE, 406th Medical General Laboratory, APO 500, San Francisco, Calif.
28. *A Case of Imported Visceral Leishmaniasis of a Dog in the United States.* RALPH E. THORSON, WILLIAM S. BAILEY, B. F. HOERLEIN, and H. R. SEIBOLD, School of Veterinary Medicine, Alabama Polytechnic Institute, Auburn, Ala.

*29. *Amebiasis in Honduras—Its Hepatic and Intestinal Complications.* MARK M. SCHAPIRO, Tegucigalpa, Honduras.

29a. *The Host-parasite Relationship in Amebiasis.* R. ELSDON-DEW, Hon. Dir., Amoebiasis Research Unit, Durban, S. Africa.

* Read by title.

2:00 P.M., November 4

SYMPOSIUM

Schistosomiasis

29b. *Introduction to Schistosomiasis Symposium.* (10 mins.) DONALD B. McMULLEN, Army Medical Service Graduate School, Washington, D. C.

29c. *Biology of the Schistosome Complexes.* (20 mins.) ROBERT E. KUNTZ, National Naval Medical Center, Bethesda, Md.

29d. *Natural History and Control of the Aquatic Snails That Transmit Schistosomes of Man.* (20 mins.) LOUIS J. OLIVIER, National Institutes of Health, Bethesda, Md.

29e. *The Biology and Control of the Amphibious Snails That Serve as Intermediate Hosts for* Schistosoma japonicum. (20 mins.) LAWRENCE S. RITCHIE, 406th Medical General Laboratory.

29f. *Immunological Aspects of* Schistosoma mansoni *Infections.* (20 mins.) JOSÉ OLIVER-GONZÁLEZ, University of Puerto Rico School of Medicine, San Juan, Puerto Rico, PRESTON M. BAUMAN, Army Medical Service Graduate School, Washington, D. C., and A. S. BENENSON, Tropical Research Medical Laboratory.

29g. *Chemotherapy in Schistomiasis.* (20 mins.) HARRY MOST, New York University College of Medicine, New York, N. Y.

The fourth scientific session was held on Friday morning, November 5, and was presided over by President Frederick J. Brady.

9:00 A.M., November 5

30. THE NINETEENTH ANNUAL CHARLES FRANKLIN CRAIG LECTURE: *Recent Advances in Our Knowledge of Dengue and Sandfly Fever.* (30 mins.) ALBERT B. SABIN, The Children's Hospital Research Foundation, College of Medicine, University of Cincinnati, Cincinnati, Ohio. Introduced by L. W. Hackett for A. C. Chandler.
31. *Serological Response of a Rural Negro Population to the Sabin-Feldman Cytoplasm Modifying Test for Toxoplasmosis.* COLVIN L. GIBSON, DON E. EYLES, NELL COLEMAN, and CLINTON

S. SMITH, National Institutes of Health, Memphis, Tenn.

32. *Observations on Some Problems Encountered in the Routine Performance of the Dye Test for Toxoplasmosis.* MORRIS GOLDMAN, Communicable Disease Center, Atlanta, Ga.
33. *A Case of Acute Toxoplasmosis Treated with Pyrimethamine and Sulfonamides.* R. F. WETTINGFELD and R. H. ROWE, Public Health Service Hospital, Memphis, Tenn. and DON E. EYLES, National Institutes of Health, Memphis Tenn.
34. *An Evaluation of the Curative Effects of Pyrimethamine and Sulfadiazine, Alone and in Combination, on Experimental Mouse Toxoplasmosis.* DON E. EYLES and NELL COLEMAN, National Institutes of Health, Memphis, Tenn.
35. *The Treatment of Toxoplasmosis of the Norway Rat with Pyrimethamine and Sulfadiazine.* FRANCES E. JONES and DON E. EYLES, National Institutes of Health, Memphis, Tenn.
36. *Recent Developments on Ocular Toxoplasmosis.* LEON JACOBS, National Institutes of Health, Bethesda, Md.
*37. *Efficacy of Sulfisoxazole Compared with Sulfadiazine and Thiocymetin Compared with Chloramphenicol on Plague in Broth Cultures and in Mice.* S. F. QUAN, U. S. Public Health Service and KARL F. MEYER, Hooper Foundation, University of California, San Francisco, Calif.
38. *The Use of Triton X-100 in Routine Giemsa Staining of Blood Parasites.* DOROTHY M. MELVIN and MARION M. BROOKE, Communicable Disease Center, Atlanta, Ga.
39. *Bacterial Diseases Transmissable from Animals to Man in the Carribean Area.* VIOLA MAE YOUNG, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

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* Read by title.

The fifth and final scientific session was held during the morning of the last day of the meeting, Saturday, November 6. President-Elect William H. Taliaferro presided.

9:30 A.M., November 6

40. *The Histopathology of the Skin in Immune and Normal Mouse Skin Exposed to Cercariae of* Schistosomatium douthitti *(Trematoda: Schistosomatidae).* IRVING G. KAGAN, University of Pennsylvania, Philadelphia, Pa. and DAVID R. MERANZE, The Albert Einstein Medical Center, Philadelphia, Pa.
41. *The Treatment of Human Schistosomiasis M. with Antimony-A, A^1-Dimercapto-Potassium Succinate (TWSb).* ERNST A. H. FRIEDHEIM, JOSÉ RODRIGUES DA SILVA, and AMILCAR VIANA MARTINS, National Health Department of Brazil, Laboratory of Ernst A. H. Friedheim, New York, N. Y., Hospital dos Servidores do Estado, Rio de Janeiro, Brazil and Medical School, Belo Horizonte, Brazil.
42. *Studies on the Biological Control of Schistosome-Bearing Snails.* ELI CHERNIN, EDWARD H. MICHELSON, and DONALD L. AUGUSTINE, School of Public Health, Harvard University, Boston, Mass.
43. *On a Non-Human Strain of* Schistosoma japonicum *in Formosa.* HSI FAN HSÜ and S. Y. LI-HSÜ, College of Medicine, State University of Iowa, Iowa City, Iowa and Iowa State Hygienic Laboratory, Iowa City, Iowa.
44. *The Collagenase of Infective Larvae of Schistosoma and Strongyloides.* ROBERT M. LEWERT and CHANG LING LEE, University of Chicago, Chicago, Ill.
45. *The Relative Egg Producing Capacity of* Schistosoma mansoni *and* S. japonicum. DONALD V. MOORE and JACK H. SANDGROUND, College of Medicine, New York University, New York, N. Y.
46. *Distribution and Prevalence of Human Schistosomiasis in Puerto Rico in 1953.* PAUL C. WHITE, JR., DAVID PIMENTEL,

FELIX C. GARCIA, and KENNETH D. QUARTERMAN, U. S. Public Health Service, San Juan, Puerto Rico.

47. *The Treatment of Ascariasis with Piperazine Citrate.* CLYDE SWARTZWELDER, JOSEPH H. MILLER, and ROBERT W. SAPPENFIELD, School of Medicine, Louisiana State University, New Orleans, La.

48. *The Treatment of* Ascaris lumbricoides *Infections with Piperazine.* HAROLD W. BROWN, School of Public Health, Columbia University, New York, N. Y.

49. *Eosinophilia and Pneumonia in Ascariasis.* RODNEY C. JUNG, School of Medicine, Tulane University, New Orleans La.

50. *Chemotherapeutic Studies on Natural Infections of* Hymenolepis nana *var.* fraterna *in Mice.* CHARLES O. BAUGHN and ALEXANDER BLIZNICK, American Cyanamid Company, Stamford, Conn.

51. *Differences in Immunity Between Strains of Cotton Rats and Their Filarial Worms.* ETTA MAE MACDONALD, J. ALLEN SCOTT, and LEROY J. OLSEN, Medical Branch, University of Texas, Galveston, Tex.

52. *Onchocerciasis in Liberia.* THOMAS A. BURCH, DONALD M. QUALLS, and HENRY J. GREENVILLE, National Institutes of Health, Bethesda, Md. and Firestone Plantations Company, Harbel, Liberia.

*53. *Effect of Host Diet on Intermediary Metabolism in* Hymenolepis diminuta. JACK W. DAUGHERTY (Introduced by ASA C. CHANDLER), The Rice Institute, Houston, Tex.

54. *Notes on the Life History of* Australorbis glabratus *Under Laboratory Conditions in Puerto Rico.* DAVID PIMENTEL and HERBERT F. SCHOOF, U. S. Public Health Service, San Juan, Puerto Rico.

55. *Pilot Project for a Medical Treatment Campaign Against Onchocerciasis Based Upon the Administration of Suramin Sodium (USP XIII). Part I. Pretreatment Survey.* THOMAS A. BURCH, National Institutes of Health, Bethesda, Md.

56. *Pilot Project for a Medical Treatment Campaign Against Onchocerciasis Based Upon the Administration of Suramin Sodium (USP XIII). Part II. Treatment and Follow-Up Examinations.* THOMAS A. BURCH, HERMAN G. AGUILAR, MANUEL BARRERA, and HERBERT T. DALMAT, National Institutes of Health, Bethesda, Md. and Ministry of Public Health and Social Welfare, Guatemala.

57. *Treatment of Elephantiasis with Cortisone in French Oceania—A Preliminary Report.* EDWARD K. MARKELL and JACQUES KERREST, School of Medicine, University of California, Los Angeles, Calif. and Institut de Recherches Medicales de l'Oceanie Francaise, Papeete, Tahiti.

58. *Effect of Quinacrine Hydrochloride and Chloroquine Diphosphate in the Treatment of 65 Human Infections with* Opisthorchis viverrini. ELVIO H. SADUN, Communicable Disease Center, Atlanta, Ga. and C. CHAMNARNKITCH, Thailand Ministry of Health, Bangkok, Thailand.

59. *Ecology of* Australorbis glabratus *in Puerto Rico. I. Relationship to Biological Environment.* DAVID PIMENTEL, PAUL C. WHITE, JR., J. W. KLOCK, and HERBERT F. SCHOOF, U. S. Public Health Service, San Juan, Puerto Rico.

*60. *The Development of the Miracidial Immobilization Reaction in Unisexual and Bisexual Infections with* Schistosoma mansoni. LAURENCE B. SENTERFIT, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

61. *The Place of the Standardized Fecal Smear for Egg Counting in Survey Work.* JOSÉ F. MALDONADO and F. RAFFUCCI, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico.

*62. *Dietary Fat and the Fat Content of* Hymenolepis diminuta. WILLIAM DACRES and JACK DAUGHERTY (Introduced by ASA C. CHANDLER), The Rice Institute, Houston, Tex.

*63. *The Action of Piperazine Against Intestinal Helminths of the Dog and*

* Read by title.

Cat. EDWIN J. DE BEER, PHILLIP H. MANN, and M. HARFENIST, The Wellcome Research Laboratories, Tuckahoe, N. Y.

SCIENTIFIC EXHIBITS

The following exhibits were displayed on the mezzanine floor of the Hotel Peabody.

"Control of Leeches and Vectors of Scrub Typhus in North Borneo." ROBERT TRAUB, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C.

"Description of the Developmental Stages of Avian and Simian Malaria." HARALD JOHNSON, Division of Medicine and Public Health, The Rockefeller Foundation, New York, N. Y.

"Intestinal Parasitic Infections in Forsyth County, North Carolina." THOMAS T. MACKIE, JANET W. MACKIE, CHARLES M. VAUGHN, NEVA N. GLEASON, BERNARD G. GREENBERG, and others.

"Arthropods, as Agents and Transmitters of Human Disease." ERNEST CARROLL FAUST, Consultant, and PAUL K. HAMILTON, Armed Forces Institute of Pathology, Washington, D. C.

"Amebiasis." ERNEST CARROLL FAUST, Consultant, Armed Forces Institute of Pathology, Washington, D. C.

"Toxoplasmosis: A Common Infection." LEON JACOBS, RALPH W. RYAN, and M. KATHERINE COOK, National Institutes of Health, Bethesda, Md.

SPECIAL EVENTS

Highlights of the meeting included a tour of the Institute of Pathology, University of Tennessee School of Medicine, and the Laboratory of Tropical Diseases, U. S. Public Health Service; the presentation of the Bailey K. Ashford Award to Joseph Greenberg (by John F. Kessel), the presentation of the Joseph Augustin LePrince Award to William H. Taliaferro for Brian G. Maegraith (by Frederick J. Brady), the presentation of the Theobald Smith Medal to George C. Shattuck (by Willard H. Wright); the presentation of the Charles Franklin Craig Lecture by Albert B. Sabin; the joint symposium on Schistosomiasis (moderated by Donald B. McMullen); and the joint dinner meeting of the Society and the Academy. E. Harold Hinman presided at the dinner session, which included the Presidential Addresses of the Society (Frederick J. Brady), and the Academy (George R. Callender).

Constitution and By-Laws of the American Society of Tropical Medicine and Hygiene*

Organized November 17, 1951, Chicago, Illinois
Incorporated Under the Laws of the State of Delaware, January 21, 1952

CONSTITUTION

ARTICLE I

Name, Origin, and Purpose

Section 1

This society shall be called the "American Society of Tropical Medicine and Hygiene."

Section 2

The Society shall be incorporated under the laws of the State of Delaware and shall be formed by the amalgamation of the American Society of Tropical Medicine, founded in 1903, and the National Malaria Society, founded in 1916.

Section 3

The purpose of this Society shall be the advancement of tropical medicine and hygiene, including medicine, nursing, engineering, entomology, parasitology, and allied specialties in this field.

ARTICLE II

Membership

Section 1

All members in good standing as of the date of amalgamation of the American Society of Tropical Medicine and of the National Malaria Society become charter members of the American Society of Tropical Medicine and Hygiene upon payment of the annual dues.

Section 2

There shall be five classes of members: Active, Honorary, Life, Emeritus, and Fellow.

Section 3

Individuals regardless of race or nationality interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care, and human welfare, may be proposed for Active Membership and may be elected as provided by the By-Laws of the Society.

Section 4

Any individual not an American citizen who has made eminent contributions to some phase of tropical medicine or hygiene shall be eligible for Honorary Membership by election

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* Articles, or Sections of certain Articles, appear in italics. This method is being used to indicate changes in the original documents (as published in the Journal, September, 1952). Amendments to the Constitution were made, according to the directives in Article V, following the tabulation of mail ballots sent to all voting members on September 14, 1954. Amendments to the By-Laws were approved by a vote of the members present at the Business Meeting, Memphis, Tennessee, November 6, 1954. It will be noted that the present Articles XI–XIV of the By-Laws are not italicized, since the numbers only were changed to permit insertion at the appropriate place of the new Article X.

as provided in the By-Laws of the Society. Such Honorary Members shall not vote or pay dues, but shall receive the official publications of the Society.

Section 5

Any Active Member may become a Life Member by payment of $200 in one sum, by which he becomes exempt from the payment of any future dues assessable during his lifetime. Each Life Member shall continue to exercise all rights of an Active Member and to receive the official publications of the Society during his lifetime.

Section 6

The Council may transfer Active Members in good standing to Emeritus Membership if the individual concerned has so requested. Such Emeritus Members shall not vote, pay dues, or receive the official publications of the Society. Eligibility for Emeritus Membership consists of retirement from active work, foreign duty, or service in the armed forces. Emeritus Members may be reinstated as Active Members upon request.

Section 7

Active members of the Society who have had not less than five years of continuous membership and who have made meritorious contributions to tropical medicine and/or hygiene may be elected to Fellowship as provided for in the By-Laws of the Society. Fellows shall pay the same dues as active members and shall continue as Fellows so long as their membership is in good standing.

ARTICLE III

Officers

Section 1

The Officers of the Society shall be a President, a President-Elect, a Vice-President, a Secretary-Treasurer, an Editor, and eight Councilors. Any member in good standing is eligible for election to office. The Officers shall constitute the Council in which the government of the Society shall be vested.

The President-Elect and the Vice-President shall be elected by the Society for a term of one year or until their successors are elected. The President-Elect at the end of his term shall become the President of the Society for the ensuing year.

The Secretary-Treasurer and Editor of Society publications shall be elected by the Council for a term of three years.

Two Councilors shall be elected by the Society at each annual meeting to serve for four years.

Terms of office in the Society shall begin at the close of the annual meeting at which the officers were elected.

If the Presidency becomes vacant during an unexpired term, the President-Elect shall become President and continue in office during the term for which he was elected. The unexpired term in office of President-Elect shall be filled by the Vice-President until the next annual meeting. The President shall appoint one of the senior Councilors to fill a vacancy in the office of the Vice-President until the next election. Vacancies among the Councilors occurring during the year may be filled by appointment by the President to serve until the next annual meeting.

Section 2

A Nominating Committee of three members representing the medical, sanitary engineering, and biologic interests of the Society shall be appointed by the Council at each annual meeting. Six months before the next annual meeting, the Nominating Committee shall submit to the Secretary the names of two or more nominees for each officer to be elected.

The Secretary shall send ballots to each member with the request that his vote be returned at least one month prior to the annual meeting. The President shall appoint a com-

mittee to count the ballots at the annual meeting so that the results can be announced in the business meeting.

ARTICLE IV

Publications of the Society

Section 1

The Society shall publish two bimonthly publications, the American Journal of Tropical Medicine and Hygiene, and Tropical Medicine and Hygiene News.

ARTICLE V

Amendment of Constitution

Amendments to this Constitution may be adopted at the annual meeting of the Society upon approval by the Officers and Councilors and upon a two-thirds affirmative vote of ballots cast, and subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. The signed proposed amendment must be in the hands of the Secretary at least six months before the annual meeting of the Society.

c. The Secretary shall mail copies of the proposed amendment to all members of the Society for their vote at least one month before the annual meeting.

d. Ballots by mail shall be counted and the results announced at the annual meeting.

BY-LAWS

ARTICLE I

The duties of the President shall be those regularly devolving on the chief executive officer. He shall preside at meetings of the Council and the Society, appoint committees as provided in the By-Laws, and he shall deliver an address at the annual meeting.

ARTICLE II

The President-Elect shall succeed the President at the annual meeting except as noted in Article III of the Constitution.

ARTICLE III

The Vice-President shall preside at meetings in the absence of the President, and he shall assist the Program Committee in duties involved with the annual meeting of the Society.

ARTICLE IV

The Secretary-Treasurer shall be the custodian of all records of the Society. He shall handle the business and finances of the Society and shall submit an annual financial report approved by a certified public accountant. He shall be responsible for the arrangement of meetings and appointment of local committees. He shall maintain the financial account of Society publications. He shall attend and record the minutes of meetings and be reimbursed from the funds of the Society for expenses incurred. He shall be bonded for a sum to be determined annually by the Council.

ARTICLE V

The Council shall manage the affairs and direct the business of the Society. It shall initiate, coordinate, and terminate the work of the committees. It shall elect the Secretary-Treasurer and Editor of publications. It shall have the power to approve the expenditure of funds and establish an annual budget for the publications of the Society.

ARTICLE VI

The Editor of Society publications with the aid of the Editorial Board is responsible for the form and content of publications of the Society. The Editorial Board shall be com-

posed of eight members to be selected and changed by the Editor subject to approval by the Council.

ARTICLE VII

Nominations for membership shall be made on the official application blank and be endorsed by two members. To be elected, the applicant must be approved by the Membership Committee.

ARTICLE VIII

Honorary Members shall be nominated by the Honorary Membership Committee, approved by unanimous vote of the Council and elected by the Society.

ARTICLE IX

The Treasurer shall open a special account for the reception of payments of Life Memberships, of which the income only may be applied to the operating expenses of the Society, and upon the death of any Life Member the amount of his membership fee shall be transferred to and become part of an endowment fund of the Society, the income of which may be expended in accordance with such regulations as the Society shall adopt.

ARTICLE X

Fellows shall be elected by a two-thirds majority vote of the Officers and Councilors of the Society.

ARTICLE XI

The annual dues of the Society shall be eight dollars which shall become due and payable the first day of January of each year. Active Members in good standing shall receive the annual volume of the Journal and published issues of the News. Any member in arrears of dues for three years shall be dropped from membership.

ARTICLE XII

Unless otherwise ordered by the Council, there shall be an annual meeting of the Society consisting of business and scientific sessions. The time and place for the annual meeting is to be determined by the Secretary-Treasurer with the approval of the Council. In the conduct of the business sessions, Robert's Rules of Order shall be the guide to the parliamentary procedure. The minutes of the annual meeting shall be published in the Journal.

ARTICLE XIII

The President shall appoint annually a Membership Committee, a Committee on The Charles Franklin Craig Lecture, and an Honorary Membership Committee, each composed of three members. The President shall appoint two members of the Society to serve on the Program Committee with the Secretary-Treasurer as Chairman, and the Editor as an ex officio member.

The Council shall appoint one Past-President every second year for a term of six years to the Committee on Award of the Walter Reed Medal composed of three Past-Presidents selected from the parent Societies or this Society; it shall appoint a member of the Society every second year for a term of six years to the Committee on the Bailey K. Ashford Award; it shall appoint every third year the Committee on the Joseph Augustin LePrince Award to consist of the President of the Society as Chairman, the immediate Past-President and the President-Elect; and it is responsible for creating, appointing, and discontinuing other committees required to transact the business of the Society.

ARTICLE XIV

The By-Laws may be amended at the annual meeting by a two-thirds vote of members present and voting, provided such amendments have been sent to the Secretary-Treasurer for circularization to the Council at least three months before the meeting.

MINUTES OF THE TWENTY-FIRST ANNUAL BUSINESS MEETING OF THE AMERICAN ACADEMY OF TROPICAL MEDICINE

The twenty-first annual meeting of the Academy was held jointly with the American Society of Tropical Medicine and Hygiene and the American Society of Parasitologists at the Peabody Hotel at Memphis, Tennessee, November 3 to 6, 1954.

A luncheon meeting of the officers and councilors of the Academy was held at noon November 4, 1954. All officers and councilors, together with six former presidents, were in attendance at the meeting and the President and the Secretary-Treasurer of the American Society of Tropical Medicine and Hygiene were present by special invitation. The agenda included the discussion of the usual business with special emphasis upon the dissolution proceedings.

The twenty-first annual dinner of the Academy was held jointly with the American Society of Tropical Medicine and Hygiene in the Continental Ballroom of the Peabody Hotel at 7:00 p.m., November 5. Dr. E. Harold Hinman acted as Toastmaster. The Theobald Smith medal of the American Academy of Tropical Medicine was presented to Dr. George C. Shattuck on behalf of the Academy by Dr. Willard H. Wright. Presidential addresses were given by Dr. Fred J. Brady on "The Outlook for Tropical Medicine" and by Brig. Gen. George R. Callender "Leonard Wood, Physician".

The business session of the Academy was held in the Louis XVI Room immediately following the adjournment of the dinner meeting. Those in attendance were: G. R. Callender (presiding), J. M. Andrews, F. J. Brady, H. C. Clark, G. R. Coatney, W. W. Cort, R. E. Dyer, E. C. Faust, Q. M. Geiman, L. W. Hackett, E. H. Hinman, H. G. Johnstone, J. F. Kessel, J. E. Larsh, T. T. Mackie, H. E. Meleney, D. B. McMullen, J. Oliver-González, C. B. Philip, A. B. Sabin, E. I. Salisbury, G. C. Shattuck, J. A. Scott, W. A. Sodeman, N. R. Stoll, W. H. Taliaferro, T. F. Whayne, W. H. Wright, M. D. Young.

The meeting was called to order by President Callender and the minutes of the Twentieth Annual Meeting, which had been published, were approved. It was recorded that "the U. S. District Court for the District of Columbia in Civil Action No. 1366-54 on July 15, 1954, adjudged, ordered and decreed that the American Academy of Tropical Medicine, Incorporated, a District of Columbia Corporation, be, and it is hereby dissolved." It was voted, subject to acceptance of the American Society of Tropical Medicine and Hygiene, that the archives, the seal of the Academy, the Theobald Smith Medal, and all financial resources and other assets of the Academy be turned over to the American Society of Tropical Medicine and Hygiene. A motion was passed that the unincorporated group of individuals formerly holding membership in the American Academy of Tropical Medicine, Incorporated, formally disband and indicate their willingness to affiliate with the American Society of Tropical Medicine and Hygiene in whatever way is acceptable to the latter association and pledge their continued devotion to the objectives of that organization. The motion was carried unanimously.

The Secretary gave his Ad Interim Report, pointing out that the Academy began the year with a complement of 77 active members, 10 emeritus members, and 9 honorary members, with a total of 96. Since the last meeting Brig. Gen. James S. Simmons, a charter member of the Academy, has died. As a result of the action of the Academy at the past annual meeting in drafting a resolution in support of the World Health Organization and the Gorgas Memorial Laboratory, Senator Alexander Wiley of Wisconsin, Chairman of the Committee on Foreign Relations of the U. S. Senate, introduced the resolution of the American Academy of Tropical Medicine and inserted a copy of the resolution into the Congressional Record of the Senate on January 15, 1954. The Theobald Smith Committee unanimously nominated Dr. George C. Shattuck to receive the Theobald Smith Medal of the American Academy of Tropical Medicine. It is recommended that an appropriate resolution be prepared in regard to the death of our charter member, Brig. Gen. James S. Simmons, and also to the local committee which has provided such splendid facilities for this meeting. As shown by the financial statement of the Secretary's office, total receipts for the year were $50.13 and expenditures were $40.38; the balance remaining was $9.75. The secretary recommended that the balance, $9.75, be utilized to transfer the archives to the office of the Secretary-Treasurer of the American Society of Tropical Medicine and Hygiene if that organization agrees to accept them. The report of the secretary including the above recommendations was accepted.

The report of the Treasurer was presented by Dr. Justin M. Andrews indicating that a balance of $140.40 remains after the payment of all expenses in connection with the dissolution of the Academy. The report was accepted and the Treasurer authorized to pay the sum of $25 to the Secretary's secretary for service to the Academy and a similar amount to the Assistant to the Treasurer of the Academy. The balance of $90.40 will be paid to the Secretary-Treasurer of the American Society of Tropical Medicine and Hygiene subject to ratification by the Society.

Prior to the conclusion of the meeting the membership adopted a recommendation originating in the meeting of the officers and councilors of the Academy which paid tribute to the energetic leadership of Dr. Earl B. McKinley in the organization and early development of the Academy. They wished also to recognize the devotion of Dr. Faust and others who continued this splendid tradition. Dr. Faust held office in the Academy for 15 years, serving as Secretary for 13. In the difficult, trying year which has just been completed the efforts of the President, the Treasurer, and the Secretary in accomplishing the dissolution of the Academy was likewise recognized.

There being no other business, the meeting adjourned at 10:30 p.m.

E. Harold Hinman, M.D.
Secretary-Treasurer

CORRESPONDENCE

New York, N. Y.
January 26, 1955

To the Editor:

Within the week I have received two just-published I.C.Z.N. Opinions that have a special interest in tropical medicine, and you may wish to bring them to the attention of readers of the AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE.

OPINION 283. Validation, under the Plenary Powers, of the generic and specific names commonly used for the Malignant Tertian Malaria Parasite and the Quartan Malaria Parasite respectively. *Opinions and Declarations Rendered by the International Commission on Zoological Nomenclature*, London, Vol. 7, Part 1, Pp. 1–226. (Issued 28 Dec. 1954.)

OPINION 312. Validation, under the Plenary Powers, of the name *Amoeba coli* as from Grassi, 1879, to be the name for the large non-dysenteric amoeba of Man and the designation of that species to be the type species of the genus *Entamoeba* Casagrandi & Barbagallo, 1895, and designation under the same powers of the name *Entamoeba histolytica* Schaudinn, 1903, to be the name for the dysenteric amoeba of Man (Class Rhizopoda) (Opinion substituted for Opinion 99). Ibid. Vol. 9, Part 1, Pp. 1–60. (Issued 17 Dec. 1954.)

Opinion 283 includes the placing in the "Official List of Generic Names in Zoology" of

a) *Plasmodium* Marchiafava & Celli, 1885 (gender: neuter) of which the type species by designation under the plenary powers is *Haemamoeba malariae* Feletti and Grassi, 1889 (the quartan malaria parasite); and

b) *Laverania* Feletti and Grassi, 1889 (gender: feminine) of which the type species by designation under the plenary powers is *Haematozoon falciparum* Welch, 1897 (the malignant tertian malaria parasite) as the generic name to be used by authors who consider the malignant tertian (i.e. aestivo-autumnal) to be generically distinct from the quartan malaria parasite.

As the title of Opinion 312 indicates, Grassi's 1879 species *coli* is now the officially designated type species for the genus *Entamoeba* Casagrandi and Barbagallo, 1895, and *Entamoeba coli* and *E. histolytica* are approved names for, respectively, the large non-dysenteric and the dysenteric amoebae of man. The opinion also places on the "Official List of Generic Names in Zoology" *Ponerамoeba* Lühe, 1909, for use by those who consider *Entamoeba histolytica* to be generically distinct from the large non-dysenteric amoeba of man.

The above constitutes a necessarily brief and incomplete statement of the rulings taken by the Commission, which need to be read in their entirety (Op. 283, pp. 3–12; Op. 312, pp. 3–6). The extensive historical analyses upon which

the decisions in both opinions have been based include significant new materials hitherto overlooked, and become valuable sourcebooks in protozoology on that account alone.

To students of taxonomic aspects it will be of further interest that since January 1954 the Commission has made great progress in clearing its desk through the publication, under Secretary Hemming, of 16 Declarations (Nos. 1–9, 13–19) and 134 Opinions (Nos. 195–329). Included are those relating to *Necator* (Op. 201), *Schistosoma* (Op. 226), *Fasciola ovata* (Op. 227), *Piroplasma annulatum* (Op. 266), *Taenia* (Op. 272), and *Leptopsylla* (Op. 285).

It might be mentioned that the effort is made to have the publications of the Commission (which has no financial endowment) pay their own way. They are sold on behalf of the I.C.Z.N. by the International Trust for Zoological Nomenclature, 41, Queen's Gate, London, S. W. 7, and may be individually purchased.

Sincerely,
Norman R. Stoll

BOOKS RECEIVED

Medical Mycology, by R. D. G. Ph. Simons (Ed.) with 36 contributors. 446 pp., illustrated. New York and Houston: Elsevier Press. 1954. $9.75.

Perspectives and Horizons in Microbiology, a symposium edited by Selman A. Waksman (Ed.) with 16 contributors. 220 pp., New Brunswick, N. J.: Rutgers University Press. 1955. $3.50.

Clear Channels—Television and the American People, by Max Wylie. 408 pp., New York: Funk and Wagnalls Company, 1954. $4.75.

Care of Laboratory Animals, by Subcommittee on Diagnostic Procedures and Reagents. 66 pp., New York: American Public Health Association. 1–19 copies 75¢; 20–99 copies 65¢; 100 and over 50¢.

Viral and Rickettsial Diseases of the Skin, Eye and Mucous Membranes of Man, by Harvey Blank, M.D. and Geoffrey Rake. 285 pp., illustrated, with 36 in color. Boston, Toronto: Little, Brown and Company. 1955. $8.50.

Diseases Transmitted from Animals to Man, by Thomas G. Hull with 24 contributors. Fourth edition: 717 pp., illustrated. Springfield, Illinois: Charles C. Thomas. 1955. $12.50.

Animal Agents and Vectors of Human Disease, by Ernest Carroll Faust. 660 pp., illustrated. Philadelphia: Lea and Febiger. 1955. $9.75.

Control of Communicable Diseases in Man, by American Public Health Association. Eighth edition: 219 pp., New York: American Public Health Association. 1955.

Mosquitoes—Their Bionomics and Relation to Disease, by William R. Horsfall. 723 pp., 206 tables. New York: Ronald Press Company. 1955. $16.00.

BOOK REVIEWS

Die Biochemie der Viren (Organische Chemie in Einzeldarstellungen, Band 5) by GERHARD SCHRAMM, Professor Dr. Phil. Max-Planck-Institute für Virusforschung, Tübingen. 276 pp., 67 illustrations. Berlin, Göttingen, Heidelberg: Springer-Verlag, 1954. Price DM 36.

Any visiting microbiologist attending congresses in Europe and others who seek medical research intelligence will hear of the ascending importance of the newly organized Max Planck Institute for Virus Research at Tübingen in the German Federal Republic. Either a fleeting visit or the recently published monograph on the biochemistry of viral agents will create respect for this new institution and its staff.

In concise and selective manner a biochemist reviews and analyzes the properties and dynamic action of viruses. He attempts to bring together the contributions of the diverse disciplines which through the past 50 years have been engaged in the elucidation of the virus as organism.

The text of the booklet describes, in the general section, the general problems related to the viruses, the methods of isolation and purification, and the size, shape and electrochemical and chemical properties of the agents. A relatively brief section mentions the immunologic properties. The virus-host relationship and mutation are considered in detail, and the book even includes a section on the control of virus infections and diseases. The arrangement under the heading "Special Part" is interesting from the standpoint of classification. It is essentially a brief description of the different viruses, divided into the following groups: crystallized plant viruses; rod-shaped viruses; size of virus developing in arthropods; bacteriophages; insect viruses. In the chapter on spherical viruses with a diameter of less than 50 mμ, affecting the homeothermic animals, the knowledge of the viruses of poliomyelitis, encephalitis, rabies and others is presented. Another section deals with the spherical agents with a diameter of more than 50 mμ. Square or irregular-shaped viruses and the psittacosis-lymphogranuloma venereum group are treated separately.

Though adequate within the scope of the book, the specialist in tropical medicine will find the section on yellow fever quite brief in contrast to the more detailed and somewhat confused presentation of the encephalomyocarditis viruses. Well-summarized and perfectly illustrated is the present-day knowledge of the vaccinia virus.

For quick, accurate orientation and for an introduction to the study of viruses the booklet, with its charts, tables and fine electron microscope pictures, fulfills its purpose. The extensive literature up to the early part of 1953 has been reviewed. One is surprised that in so up-to-date a list the 1948, rather than the 1952, edition of the Rivers book on viral and rickettsial infections of man was the one consulted.

K. F. MEYER

Explored Areas of Arthropod-borne Viral Infections (Yellow Fever and Dengue Excepted)

We are indebted to the American Geographical Society for another map in their series on the world distribution of various diseases or infections. The latest map represents a very important contribution since, for the first time, it is possible to see at a glance the areas in which arthropod-borne viral infections occur. The extent to which arthropod-borne viruses are encountered over the world comes as a surprise even to those familiar with the literature, for here in the map we have brought together for the first time the findings and observations recorded in a rather comprehensive and scattered literature; the selected bibliography, which is quite extensive, contains a number of review papers and thus obviates the necessity of going back to many original papers.

In view of the excellence and the clarity with which the data are presented, one hesitates to bring up what might be regarded as minor flaws. Thus, in Maps 6, 7, and 8 of the 10 presented, the legend makes a distinction as to whether the area of occurrence of an arthropod-borne agent is based on the presence of antibodies or on actual isolation of the agent. In the other maps, however, the legends referring to "virus occurrence in animals", and to "virus present in animals", for example, make no distinction as to whether this rests on antibody surveys, on virus isolation, or both. Thus, in Brazil, two areas are labelled in two different maps as "virus occurrence in animals" and as "virus present in animals" respectively. However, in the Pecanha area the presence of Eastern equine encephalomyelitis virus in that region was based on the finding of EE antibodies in horses. In the Ilheus area, however, the so-called Ilheus virus was isolated from monkeys.

In the box labelled "remarks", there is some uncertainty as to what is meant by certain statements. In the introductory sentence it is stated that areas of known occurrence of arthropod-borne viral infections are indicated in animals by tints and in human beings by symbols. Occurrence is revealed "by clinical diagnosis, isolation of virus and detection of antibodies. Only when the virus has been isolated is the cause of the occurrence proved beyond doubt." Dealing with the United States it is stated that the work of Wooley and Armstrong in 1934 indicated that St. Louis encephalitis virus was present in California, Colorado, Illinois, Minnesota, Missouri, Nebraska, New York, Ohio, Oregon, Tennessee, Texas and Utah. However, it is stated that the validity of these results is subject to discussion, since only in St. Louis, Kansas City (Kansas), the Yakima Valley of Washington, and Arizona has the virus been isolated or the infection really proved to be due to the virus by an increase in serum antibodies between two bleedings. It is not entirely clear whether the reference is to humans or to animals or to both. If to humans, then California should be included as an area in which the St. Louis encephalitis virus has been isolated. Nevertheless, the map represents an excellent end product of a great deal of devoted labor and is recommended to all interested in, or concerned with, arthropod-borne viral infections.

E. H. LENNETTE

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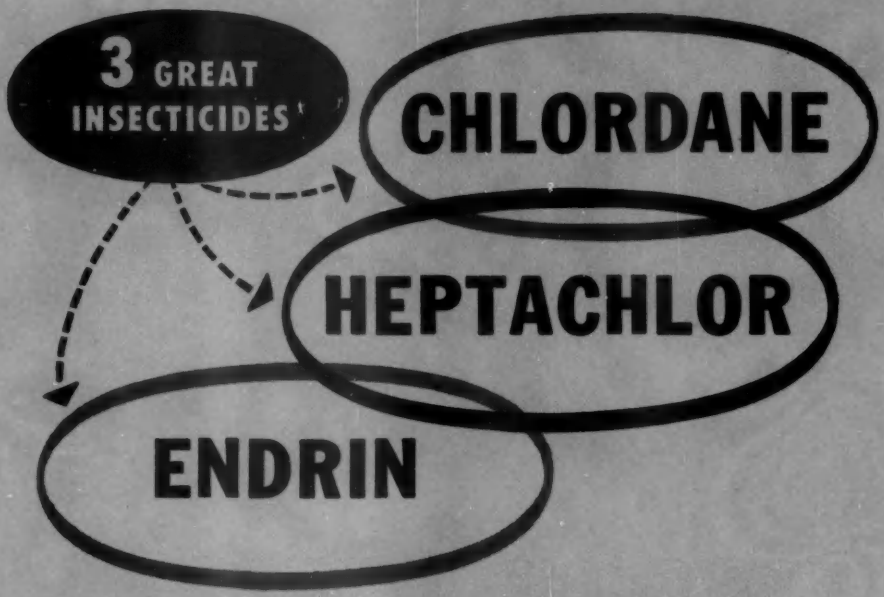

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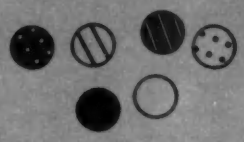

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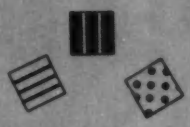

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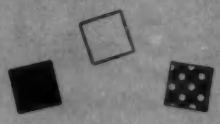

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